

Cys + COSY Stability Under Ligation Conditions

1. In the absence of a thioester peptide

H-CGFRVREFGDNTA-COSH MW=1487.6

6M GU·HCL, 0.1M NaPi, 0.5% thiophenol, room temperature, overnight

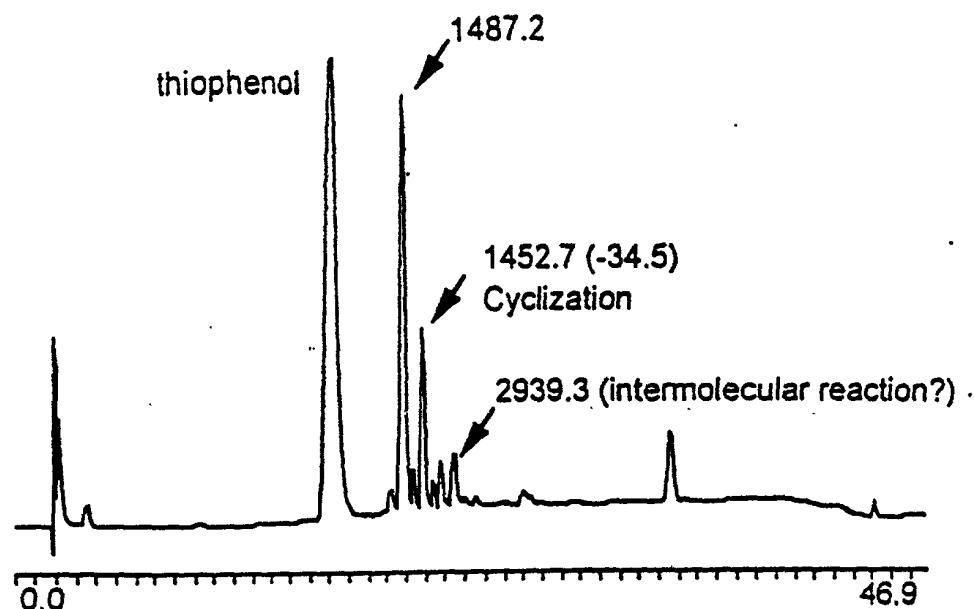


FIG. 2A

2. In the presence of a thioester peptide

H-CGFRVREFGDNTA-COSH MW=1487.6 + *H*-DSVISLSGDH-SPAL MW= 1230.2

MW of Ligation product = 2498.7

6M GU·HCL, 0.1M NaPi, 0.5% thiophenol, room temperature, overnight

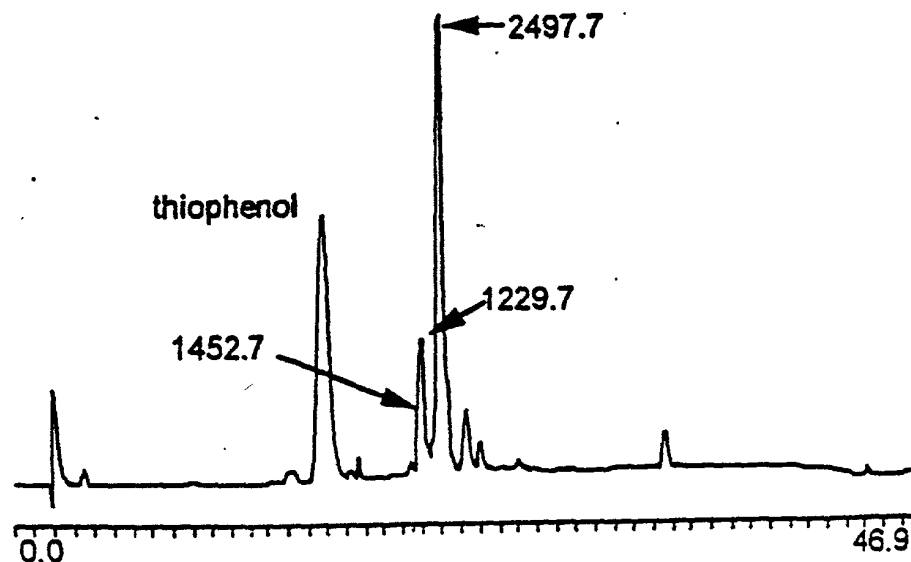


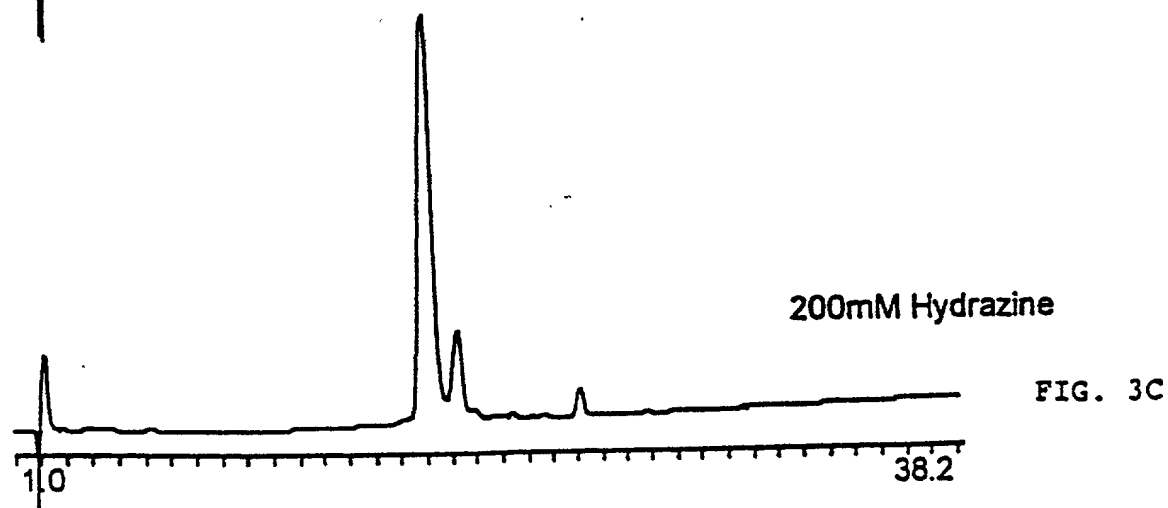
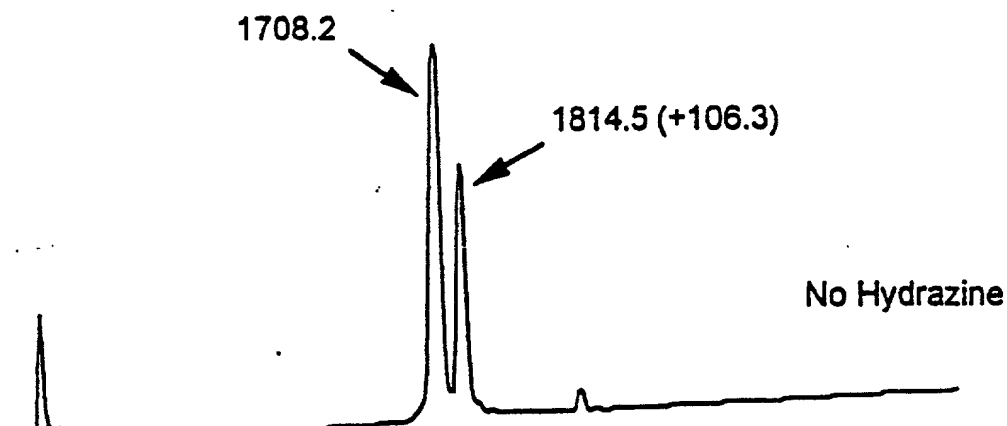
FIG. 2B

MSC Removal Experiments

MSC-CTSAGPHFNPLSRKHG-OH MW=1859.1

H-CTSAGPHFNPLSRKHG-OH MW=1708.9

Aliquot of peptide in 6M Gu·HCl, 0.1M
NaPi, pH 7.5 was diluted into 1N NaOH for
two minutes, quenched with 1N HCl



MSC Removal Experiments (Cont'd)

Lev-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSH

MW=4022.4

H-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSH

MW=3745.1

Aliquot of peptide in 6M Gu•HCl, 0.1M NaAc, pH 4.6 was diluted into 6M Gu•HCl, 0.1M NaAc, pH 14 for two minutes, quenched with 6M Gu•HCl, 0.1M NaAc, pH 2.0

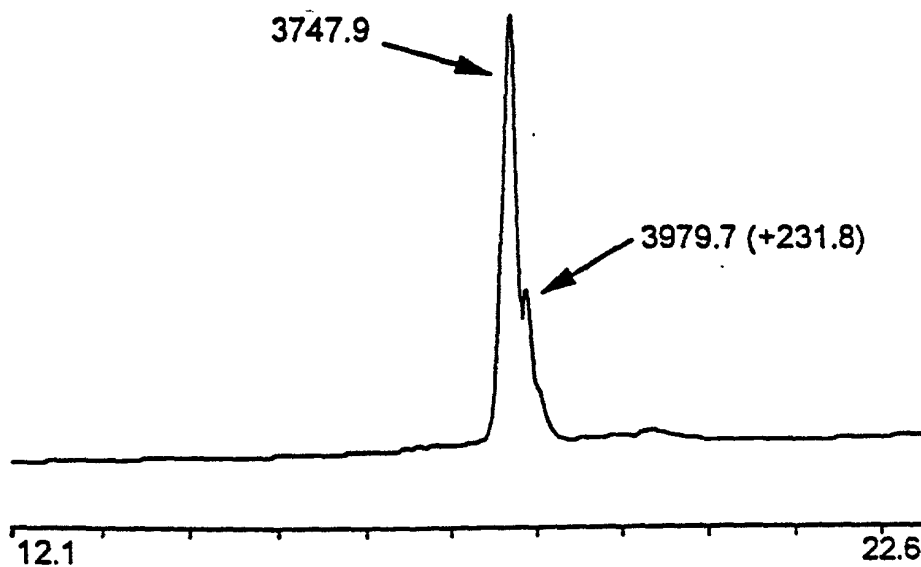


FIG. 4

Resin Preparation

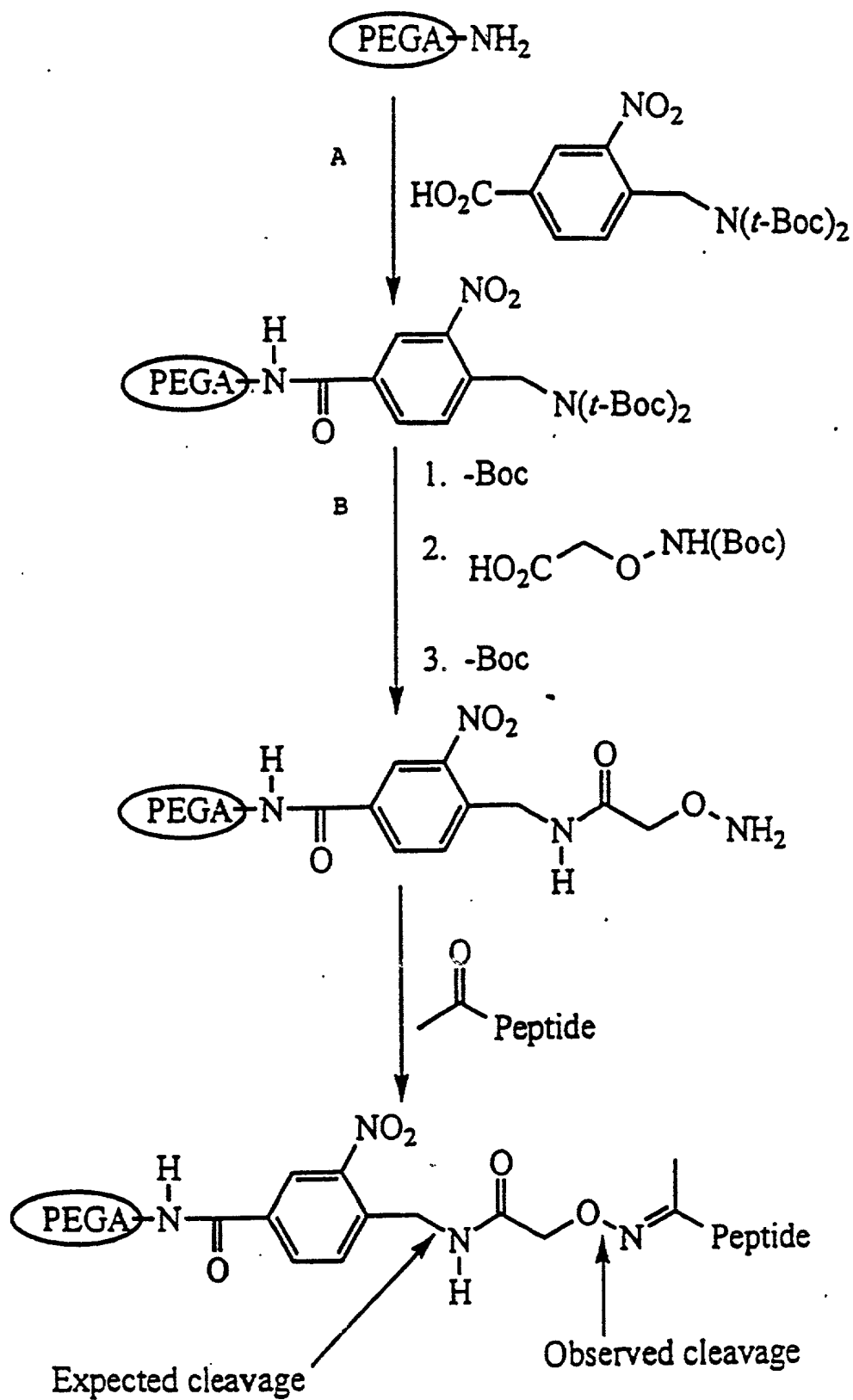
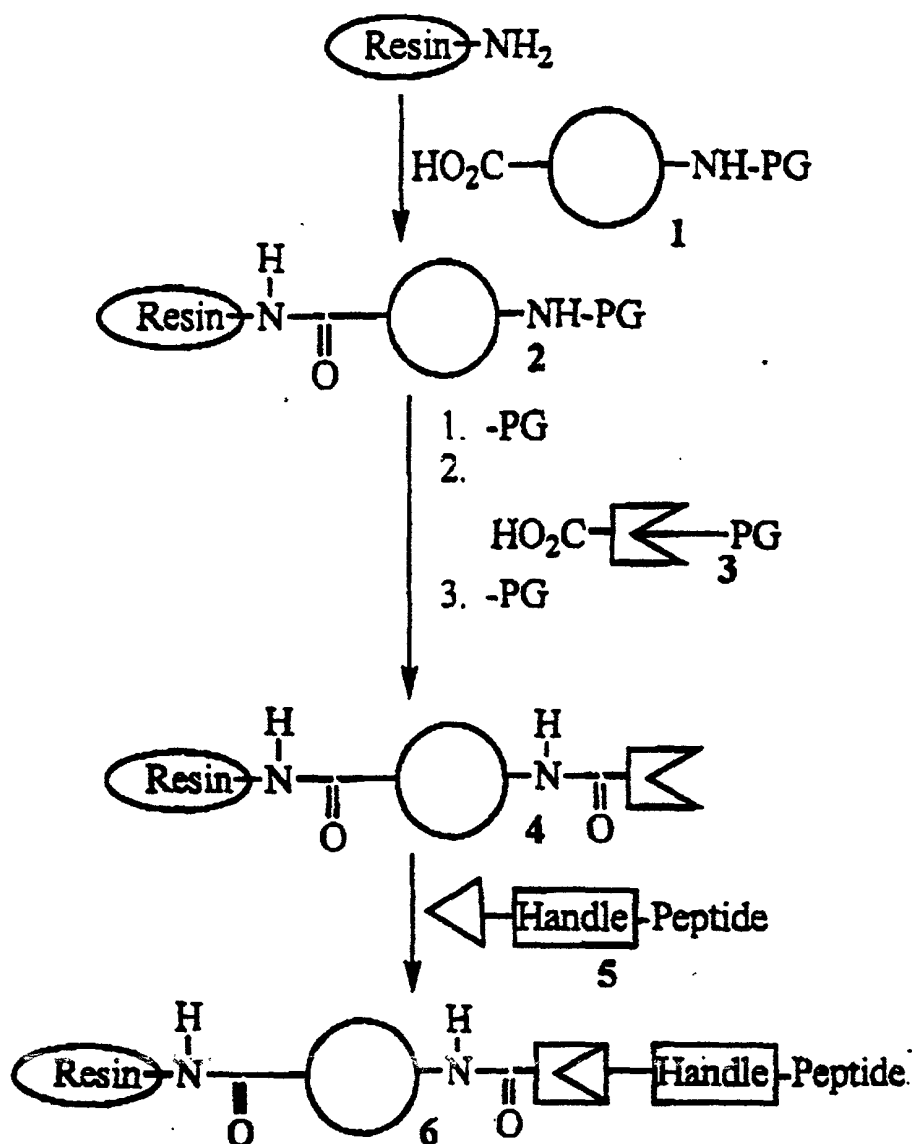


FIG. 5A

Resin Preparation



$\text{HO}_2\text{C}-\text{Circle}-\text{NH}-$ = cleavable linker used for monitoring with Maldi, Electrospray Mass Spec, etc...

PG = protecting group

$\text{HO}_2\text{C}-\text{Zigzag}$ = functional group added to resin to couple with peptide

$\text{Triangle}-\text{Handle}-\text{Peptide}$ = peptide functionalized with 1) a cleavable handle for release of peptide/protein from the resin at completion of synthesis and 2) functional group to couple to resin

FIG. 5B

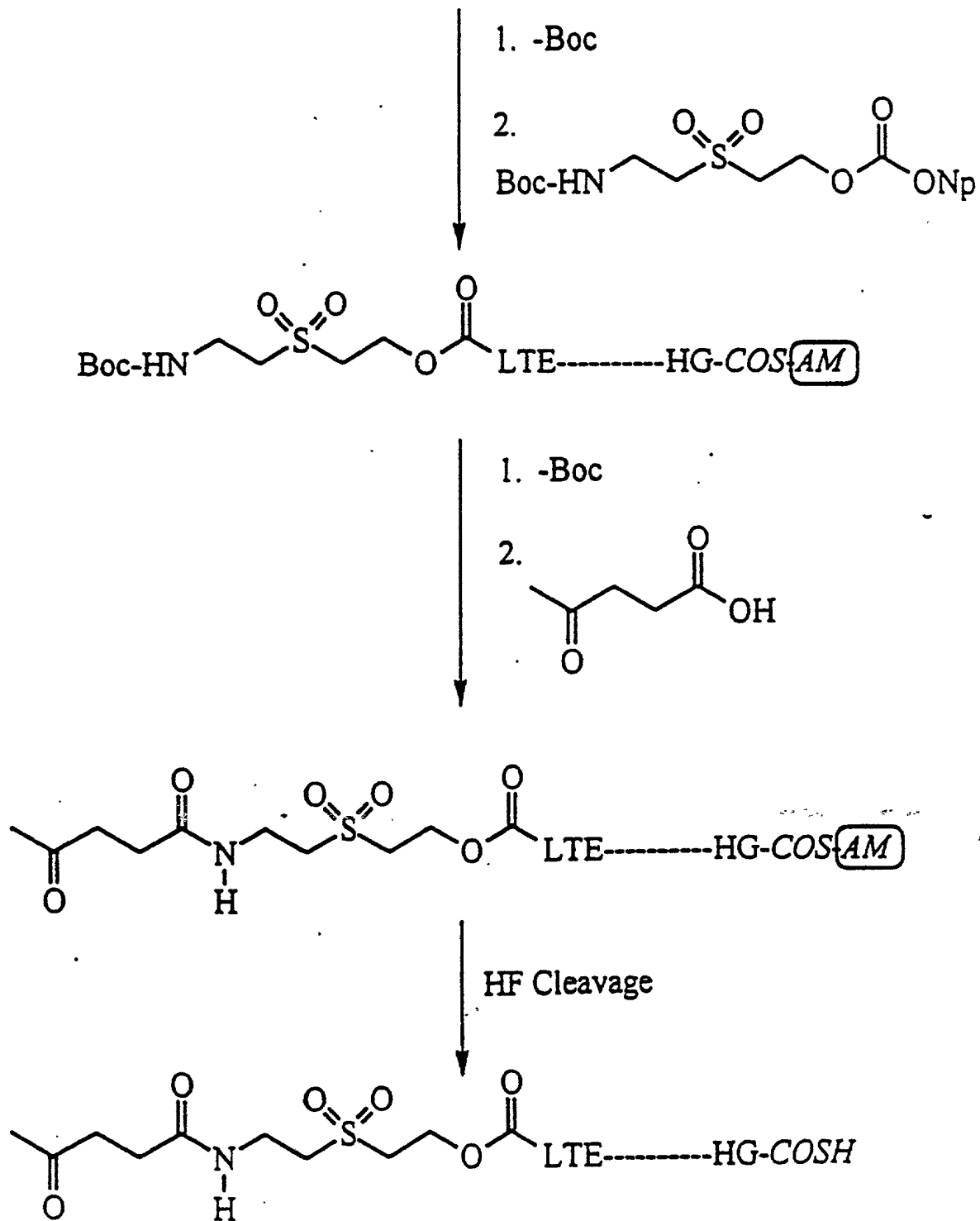
[illegible]

FIG. 6

Polymer-Supported Ligation on PEGA

Lev-*MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSH*(1)
+ Resin-PCL-ONH₂

↓ 1. pH 4.6, 6M Gu•HCl, 0.1 acetate

Resin-PCL-oxime-MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSH(1)

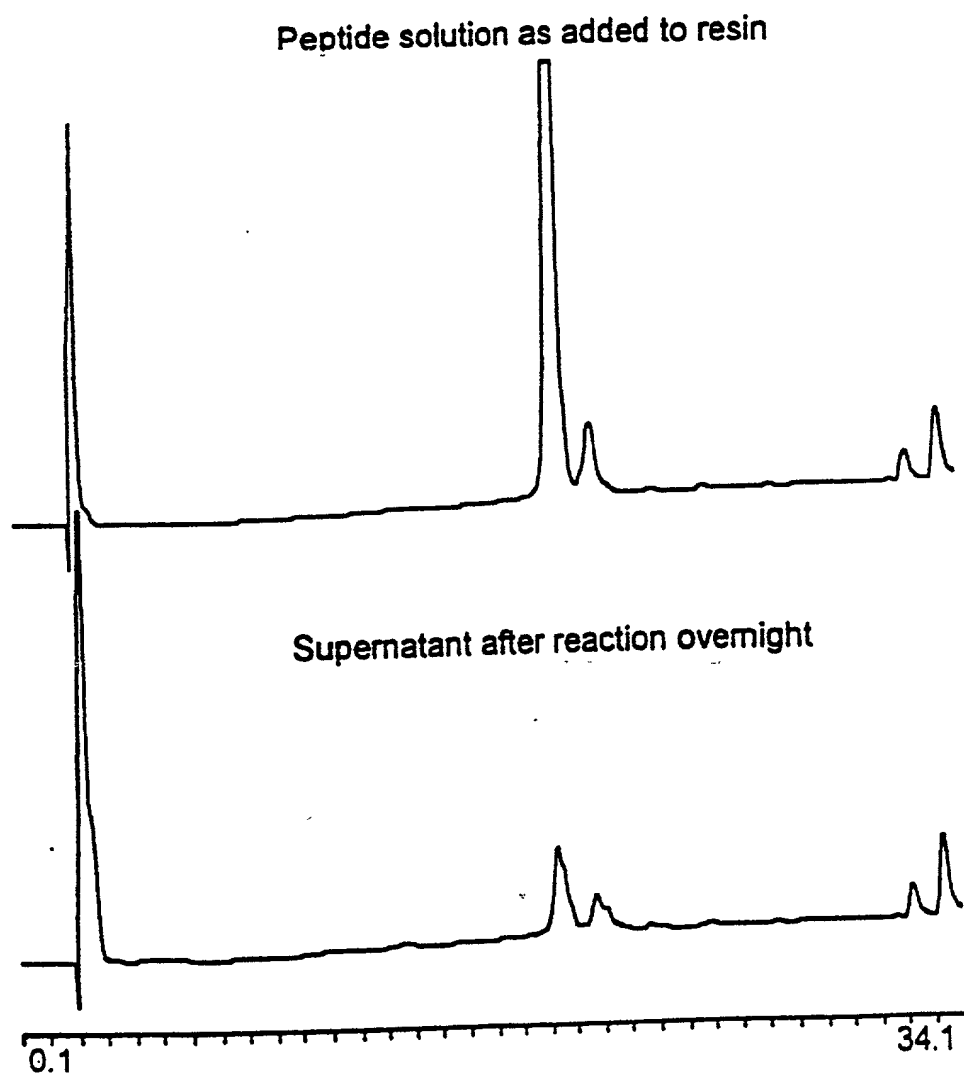


FIG. 71

A

FIG. 71

B

Polymer-Supported Ligation on *Iseo*

Lev-*MSC*-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSH(1)
+ Resin-PCL-ONH₂

↓ 1. pH 4.6, 6M Gu·HCl, 0.1 acetate

*Resin-PCL-oxime-MS*C-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSH(1)

Peptide solution as added to resin

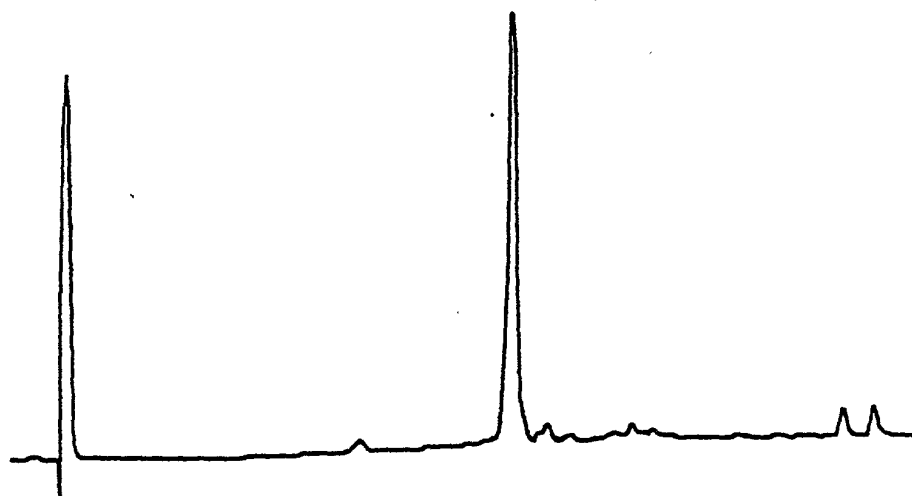


FIG. 8A

Supernatant after reaction overnight

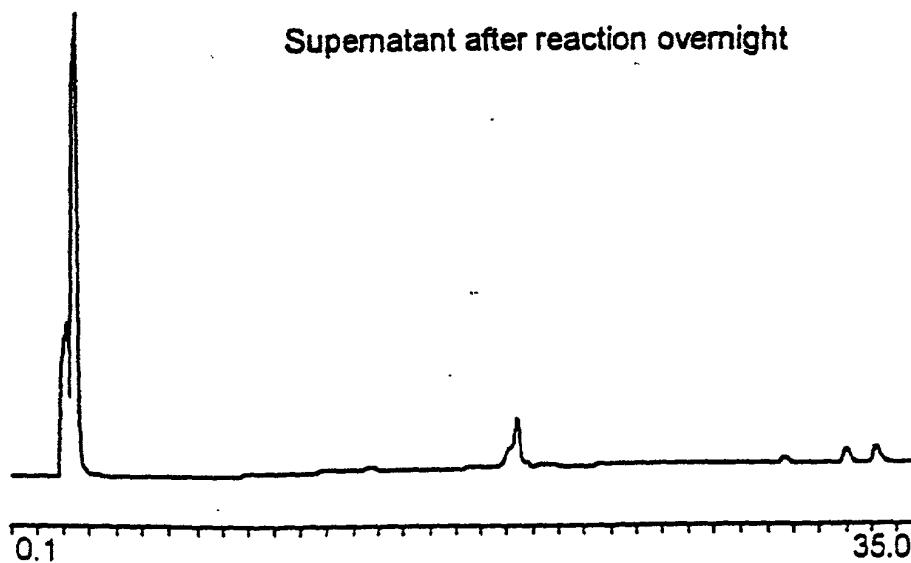


FIG. 8B

Polymer-Supported Ligation on IscO

Lev-*MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSH* (1)
+ Resin-PCL-ONH₂

↓ 1. pH 4.6, 6M Gu·HCl, 0.1 acetate

Resin-PCL-oxime-*MSC-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSH* (1)
Maldi Mass = 4022, Base Cleavage Mass = 3745

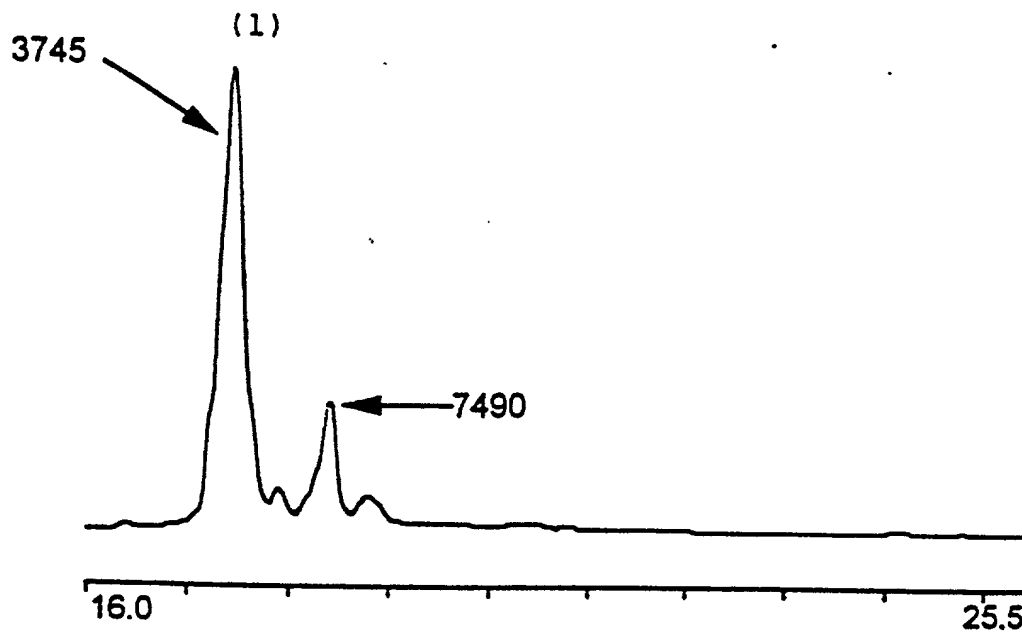


FIG. 9A

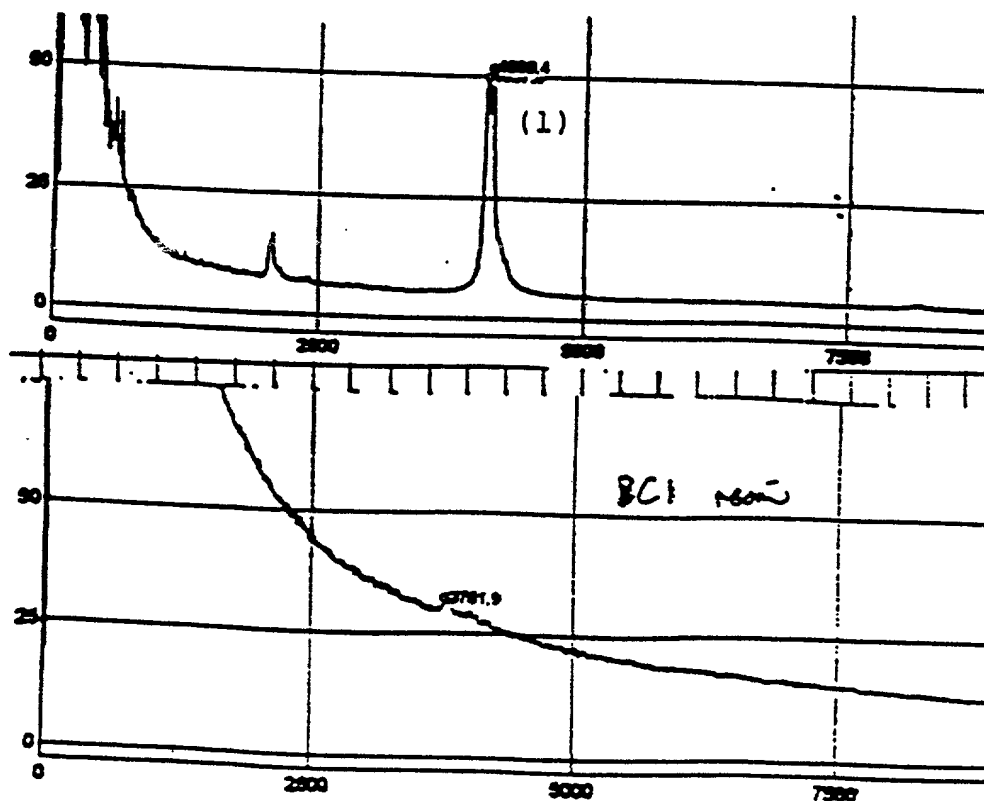


FIG. 9B

FIG. 9C

Polymer-Supported Ligation on Isco

Resin-PCL-oxime-~~MSC~~-L₁EGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG-COSAc (1)

Maldi Mass = 4080, Base Cleavage Mass = 3729

+ H-CGFRVREFGDNTA-COSH (2)

↓ 3. pH 7.5, 6M Gu•HCl, 0.1M phosphate, 0.5% thiophenol

Resin-PCL-oxime-~~MSC~~-LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHGCGFRVREF-GDNTA-COSH (1+2)

Maldi Mass = 5476, Base Cleavage Mass = 5199

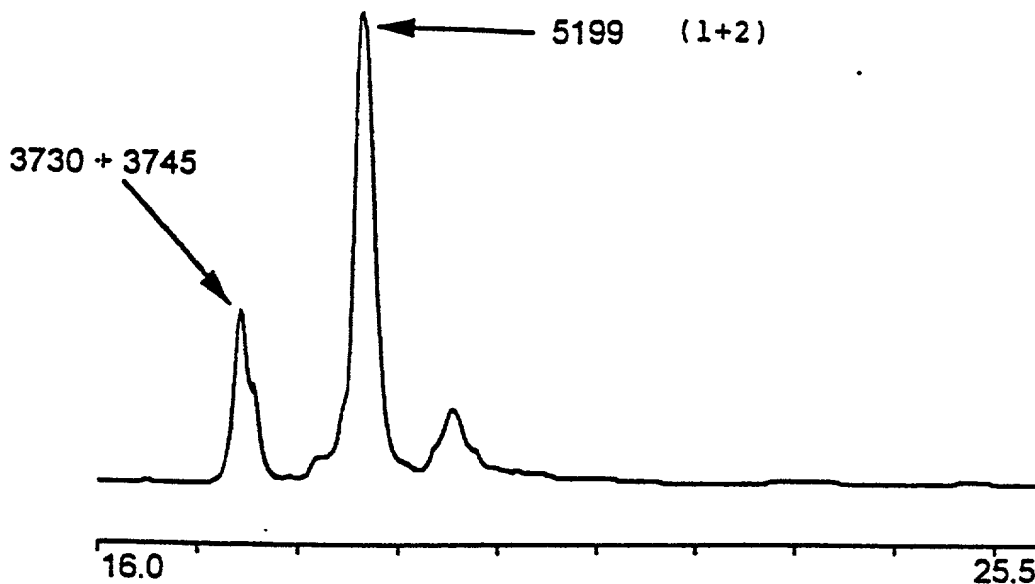


FIG. 10
A

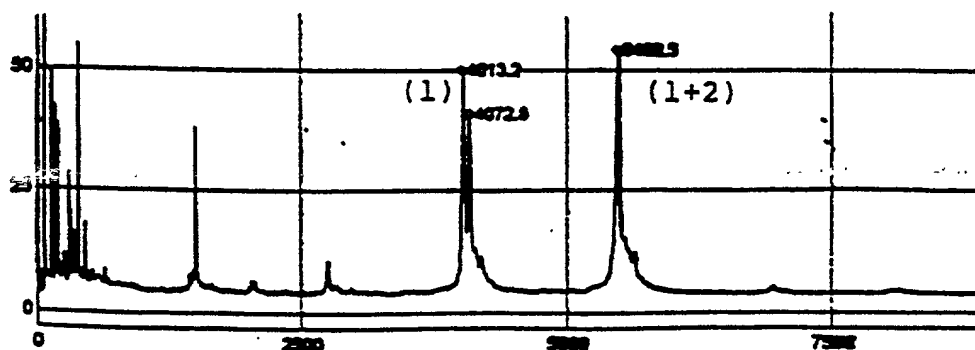


FIG. 10
B

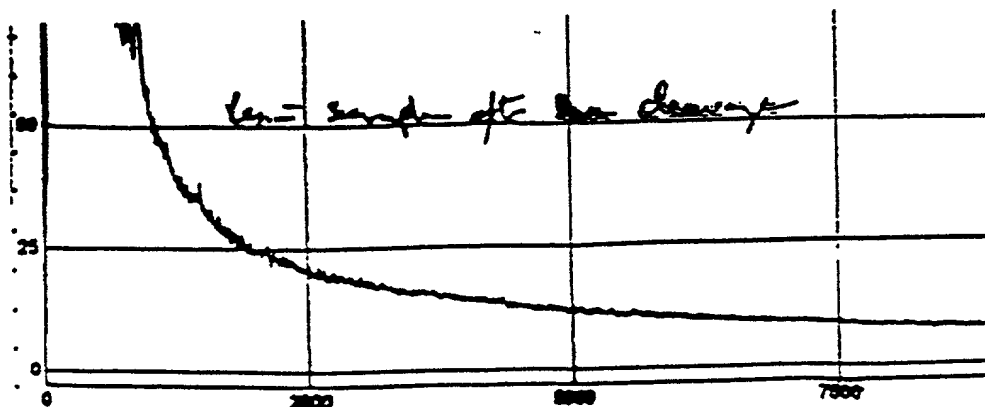


FIG. 10
C

Polymer-Supported Ligation on Isco

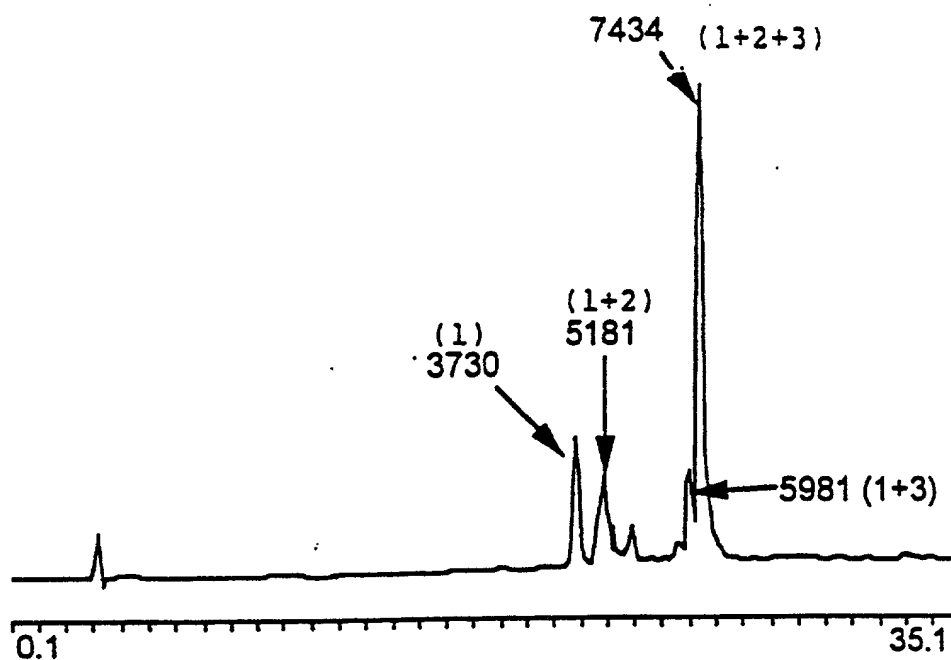


FIG. 11

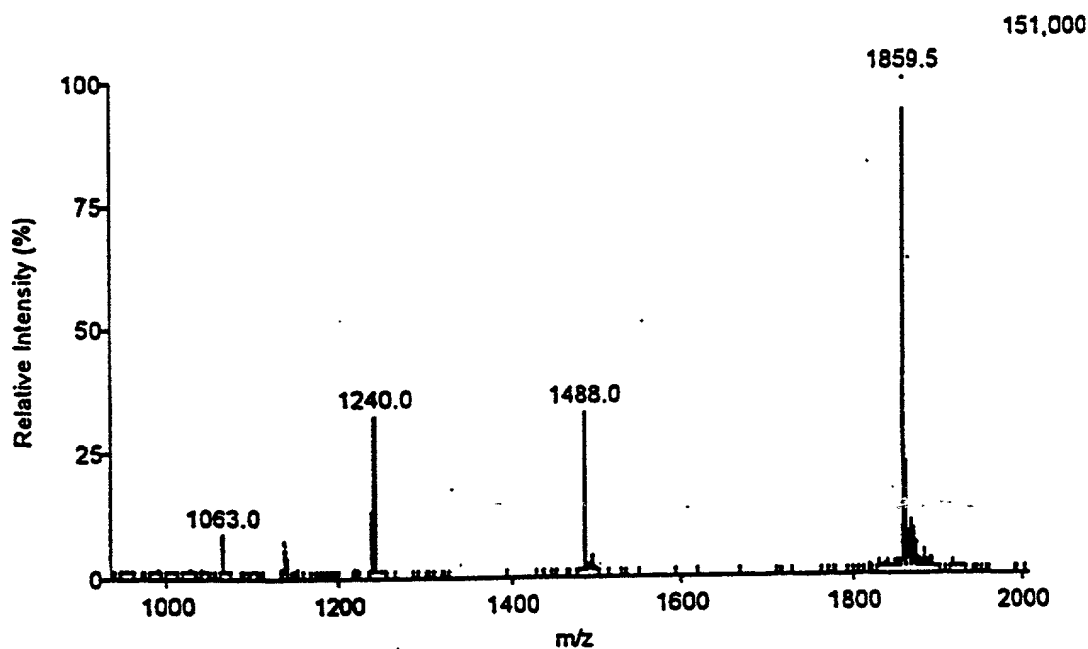


FIG. 12A

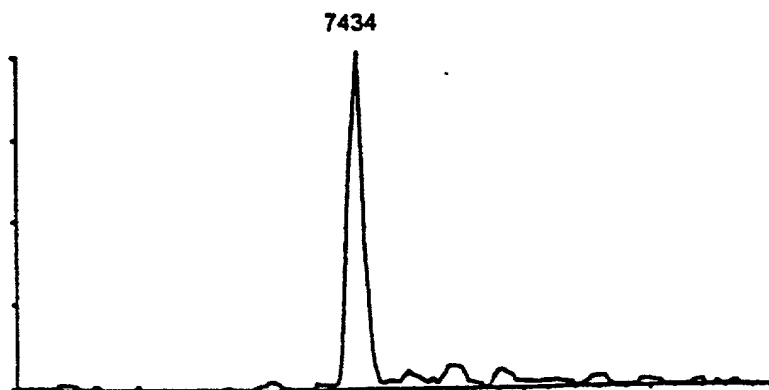


FIG. 12B

Polymer-Supported Ligation on PEG No photocleavable linker

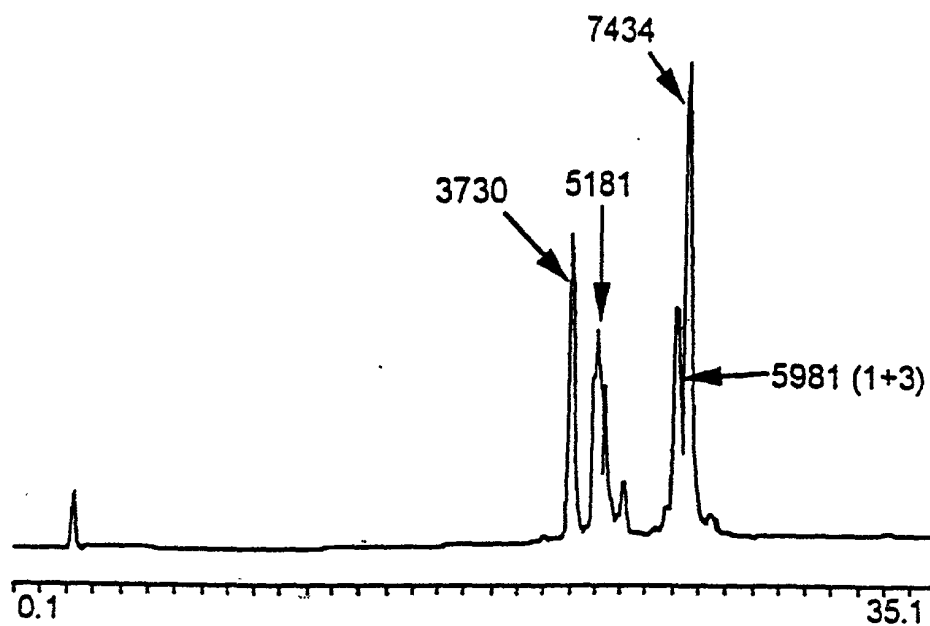


FIG. 13

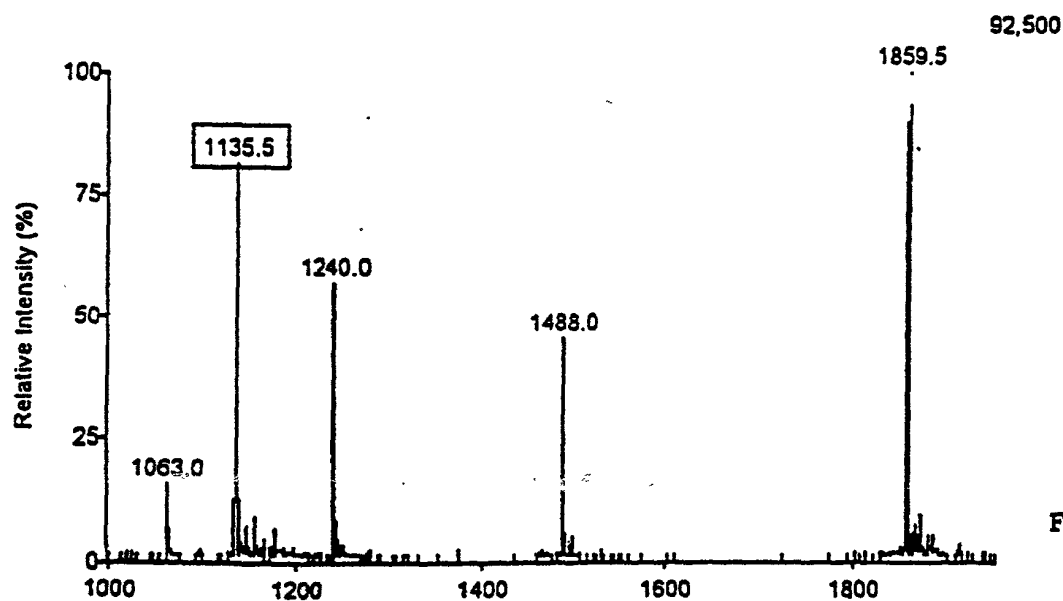


FIG. 14A

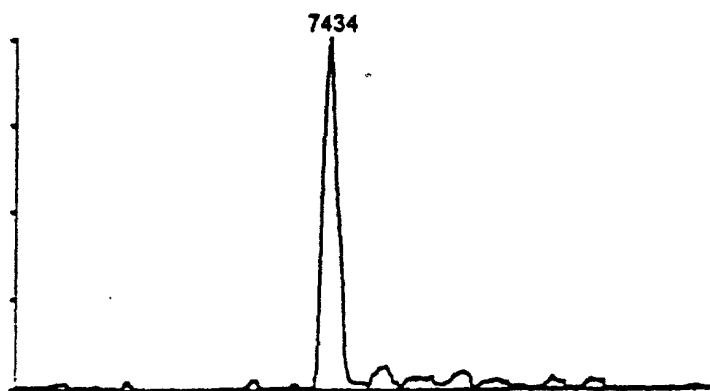


FIG. 14B

On Resin Purification

Solution processing

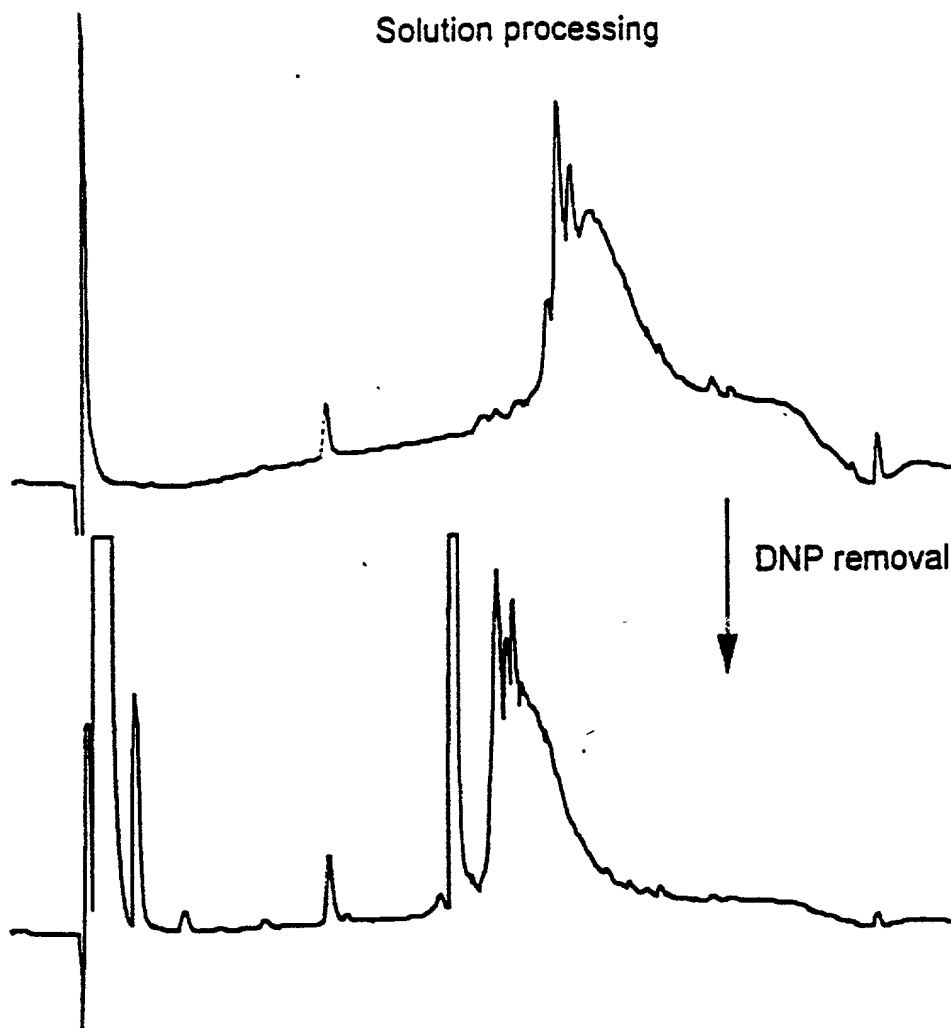
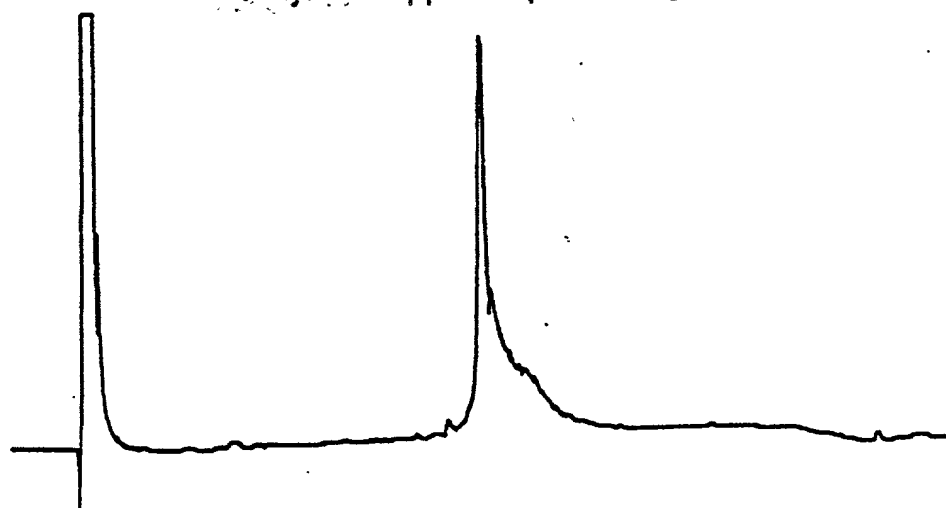


FIG. 15A

FIG. 15B

0.0 46.8

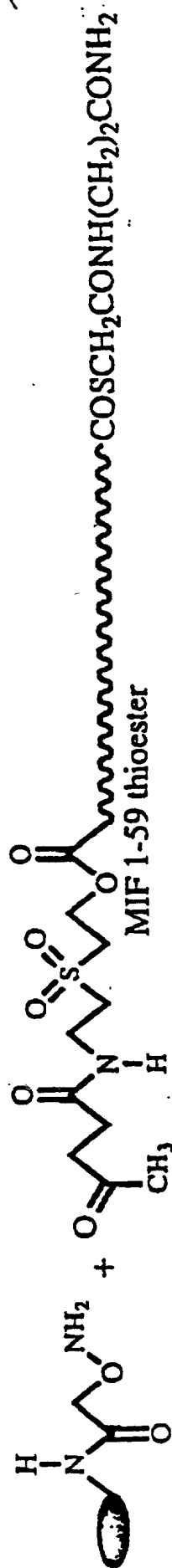
Polymer-supported processing



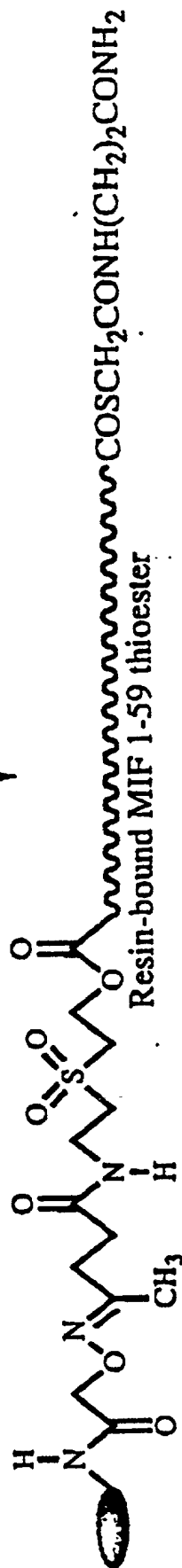
0.1 46.8

FIG. 15C

Synthesis of MIF by Solid Phase Native Ligations



Step #1: coupling MIF 1-59 to solid support
 pH 4.6, 24 hrs



Step #2: first ligation to form MIF 1-80

Cys-COS⁻, pH 7.5, 24 hrs
 MIF 60-80 thioacid

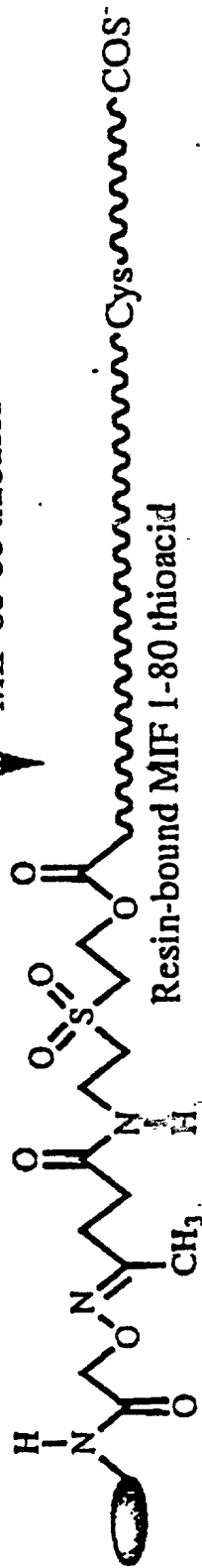


FIG. 16A

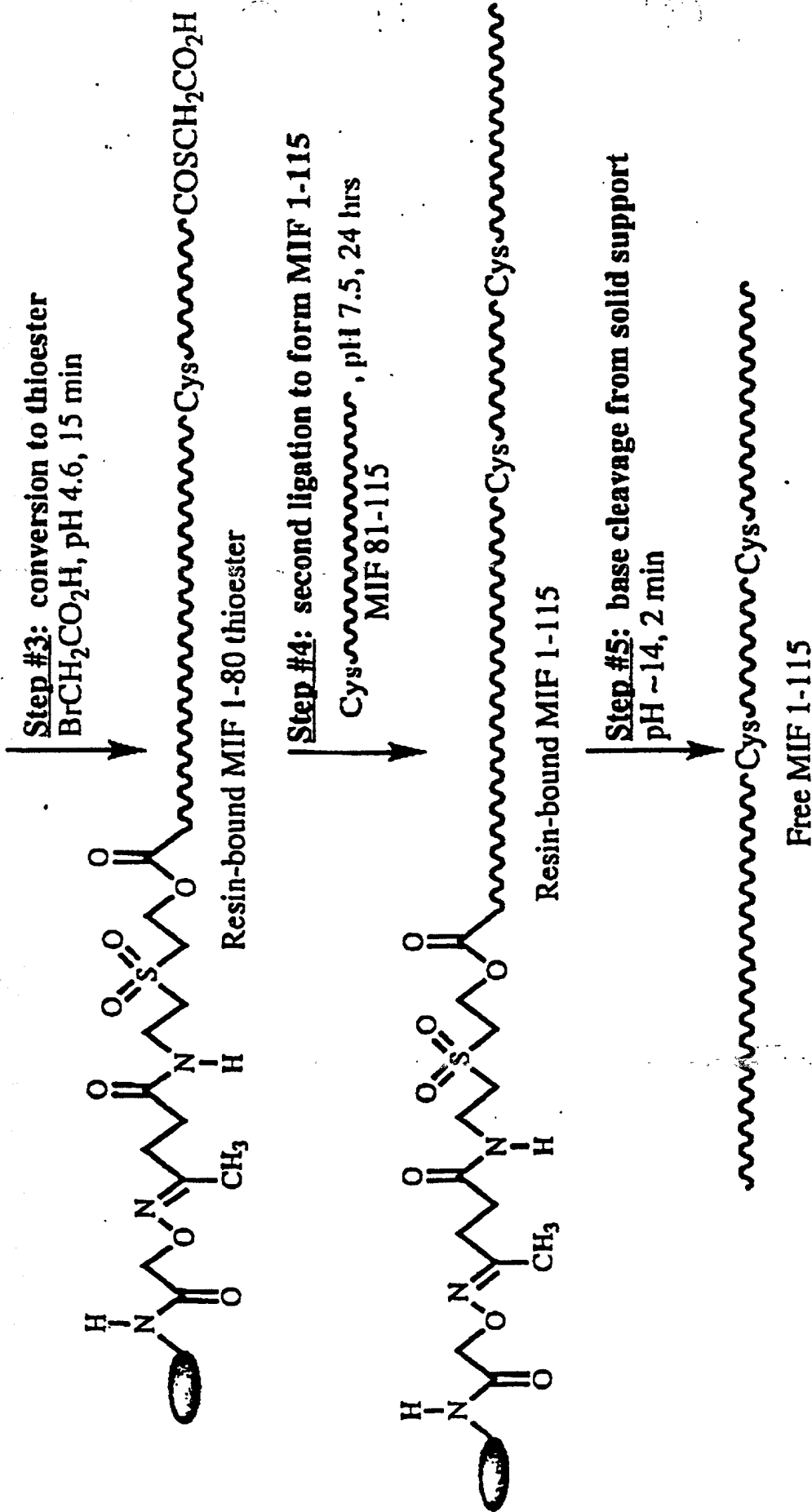


FIG. 16B

Modification of N-terminal Peptide Segment and Solid Support

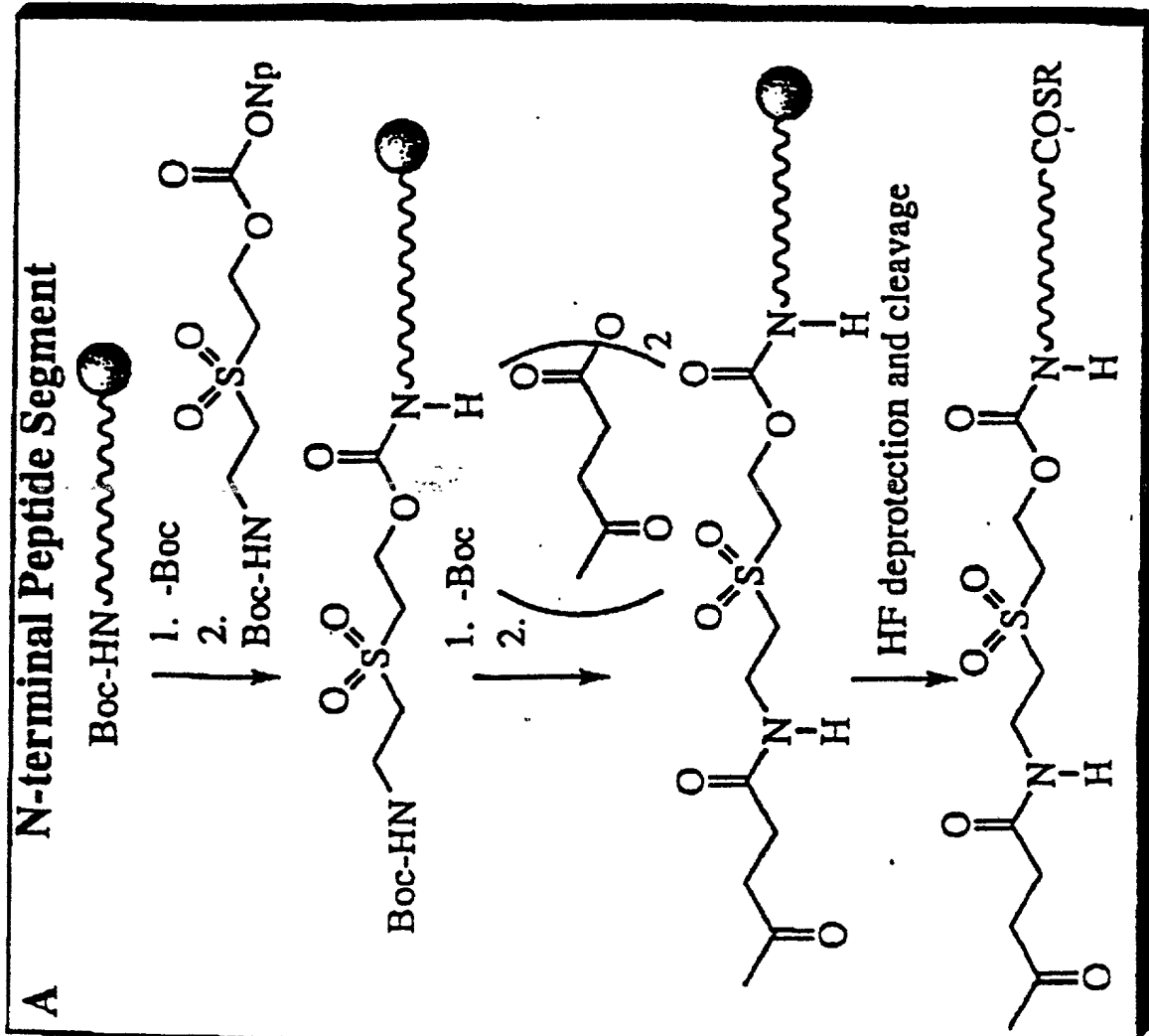


FIG 17A

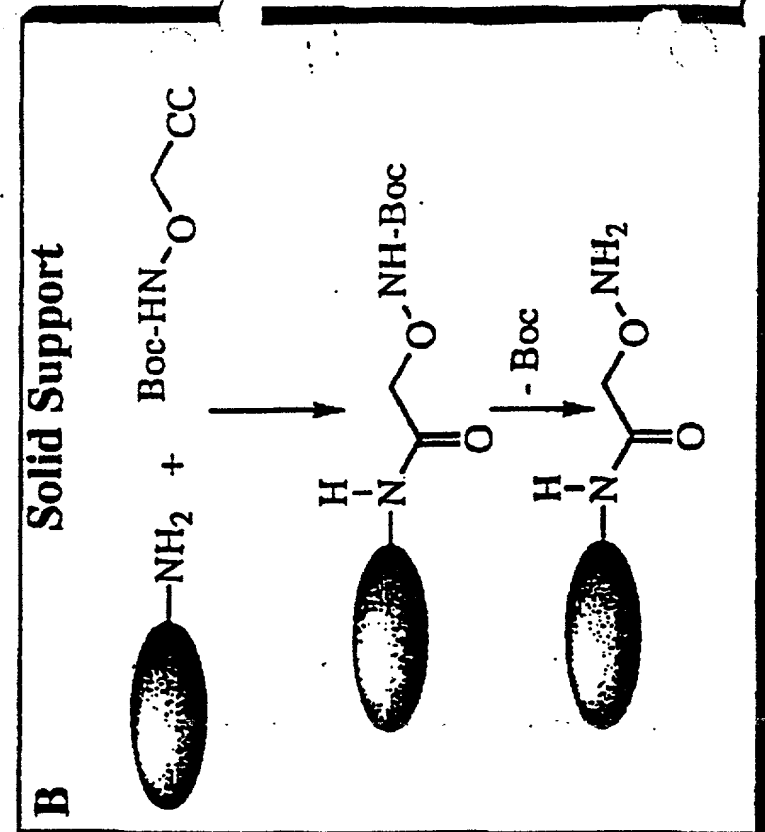
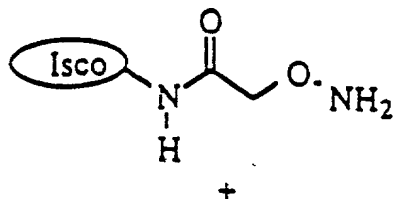


FIG. 17B

Coupling of MIF 1-59 to Solid Support



Ketone-MS handle-Met¹-MIF 2-58-Leu⁵⁹-SAc-βAla-CO₂H

#1

Isco-Oxime-MS handle-Met¹-MIF 2-58-Leu⁵⁹-SAc-βAla-CO₂H
 Expected base cleavage mass = 6271

FIG. 18A

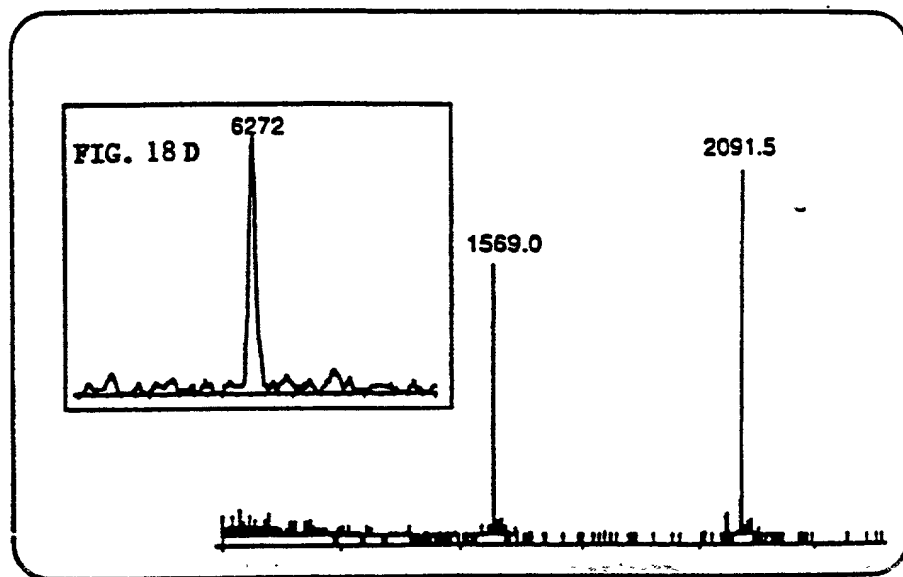


FIG. 18C

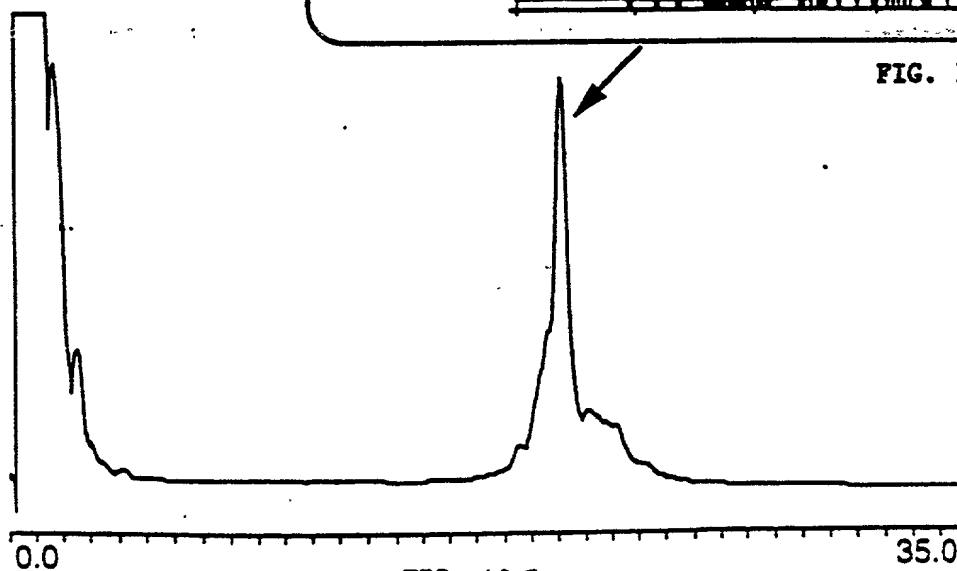


FIG. 18B

Ligation to form MIF1-80

Isco — Oxime-MS handle-Met¹-MIF 2-58-Leu⁵⁹-SAc-βAla-CO₂H

#2
↓
Cys⁶⁰-MIF 61-79-Leu⁸⁰-COSH

Isco — Oxime-MS handle-Met¹-MIF 2-79-Leu⁸⁰-COSH
Expected base cleavage mass = 8502

FIG. 19A

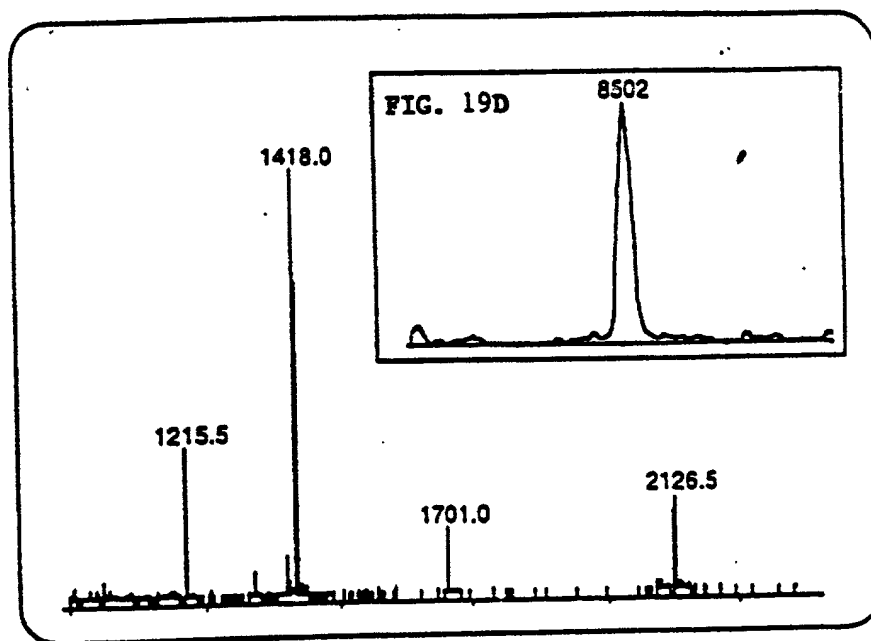


FIG. 19C

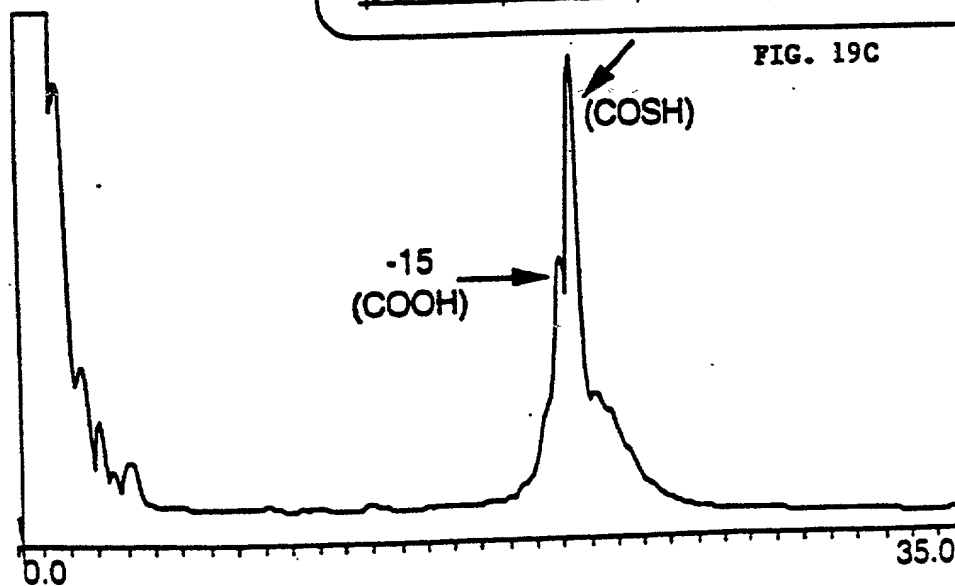


FIG. 19B

Ligation to form MIF 1-115

Isco — Oxime-MS handle-Met¹-MIF 2-79-Leu³⁰-COSAc

#4
 ↓ Cys⁸¹-MIF 82-114-Ala¹¹⁵-CO₂H
 6M Gu•HCl, 0.1 M Na Pi, 0.5% thiophenol
 0.15 M Methionine, pH 7.5

Isco — Oxime-MS handle-Met¹-MIF 2-114-Ala¹¹⁵-CO₂H
 Expected base cleavage mass = 12450

FIG. 20A

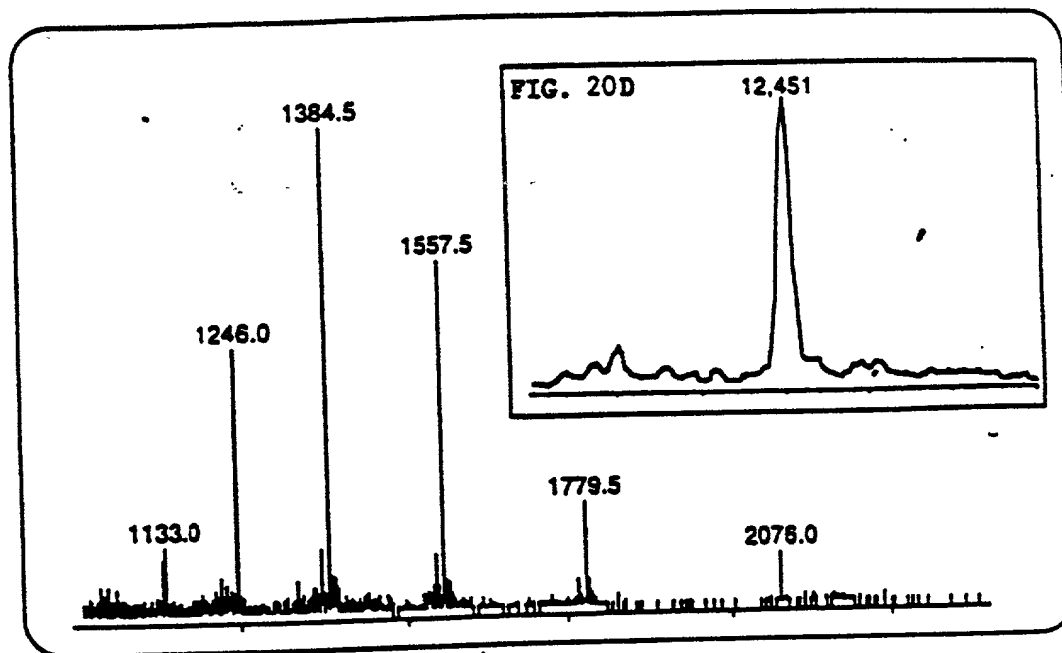


FIG. 20 C

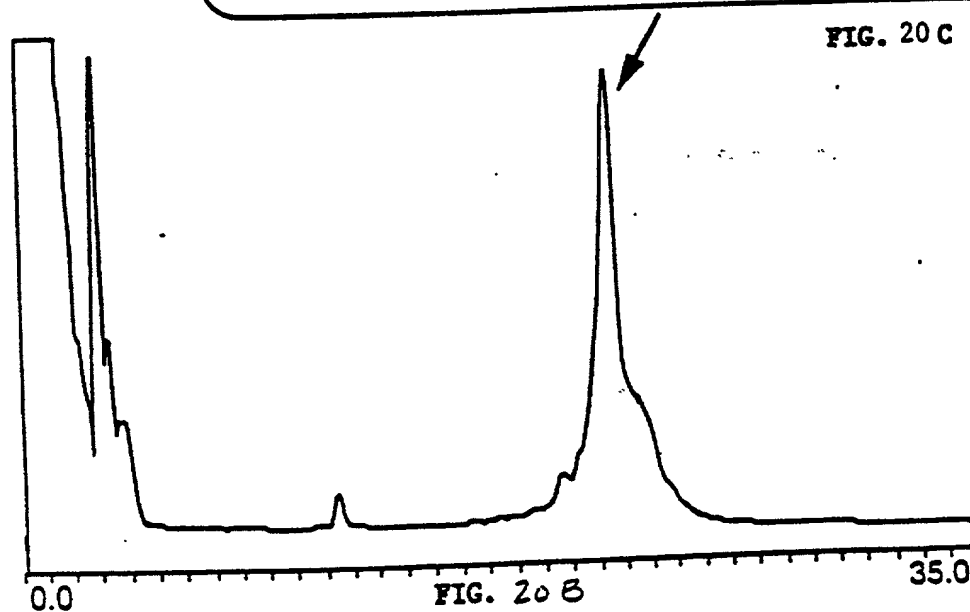


FIG. 20 B

Solid Phase Chemical Ligations in the C- to N-terminal Direction

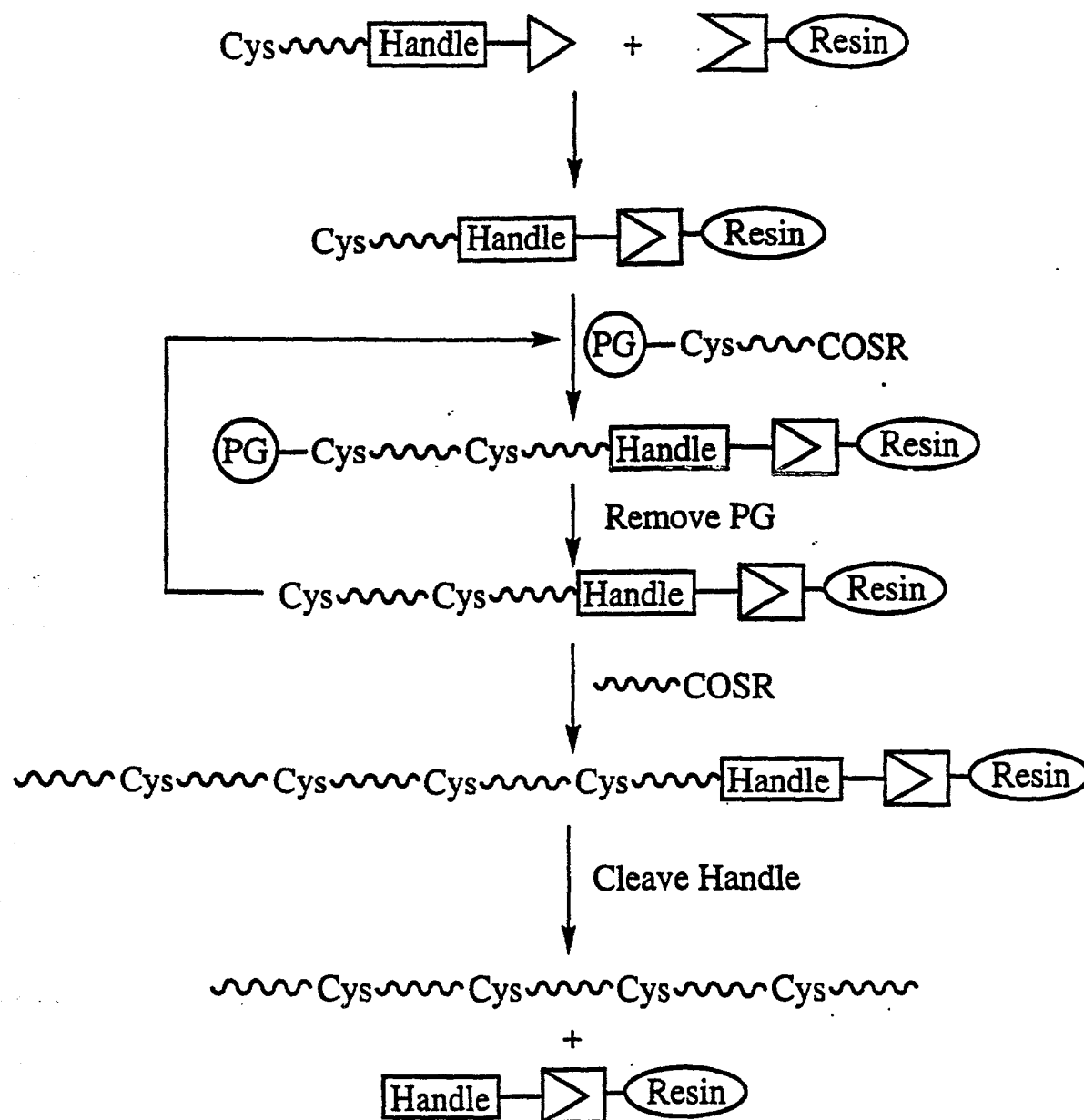
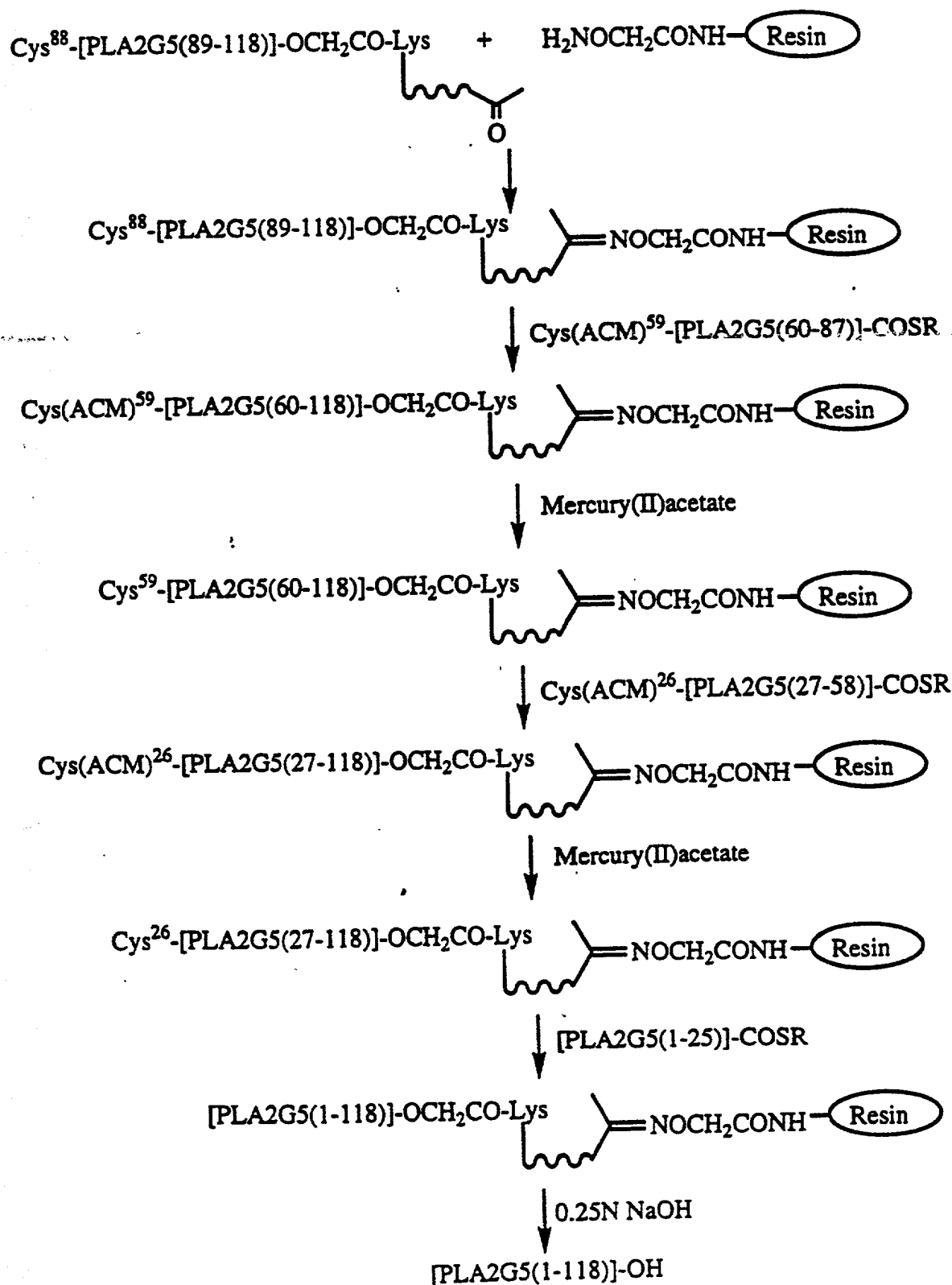


FIG 21

Solid Phase Chemical Ligations in the C- to N-Terminal Direction Synthesis of Phospholipase A2, Group 5 (PLA2G5)



Synthesis of Cam ester derivative

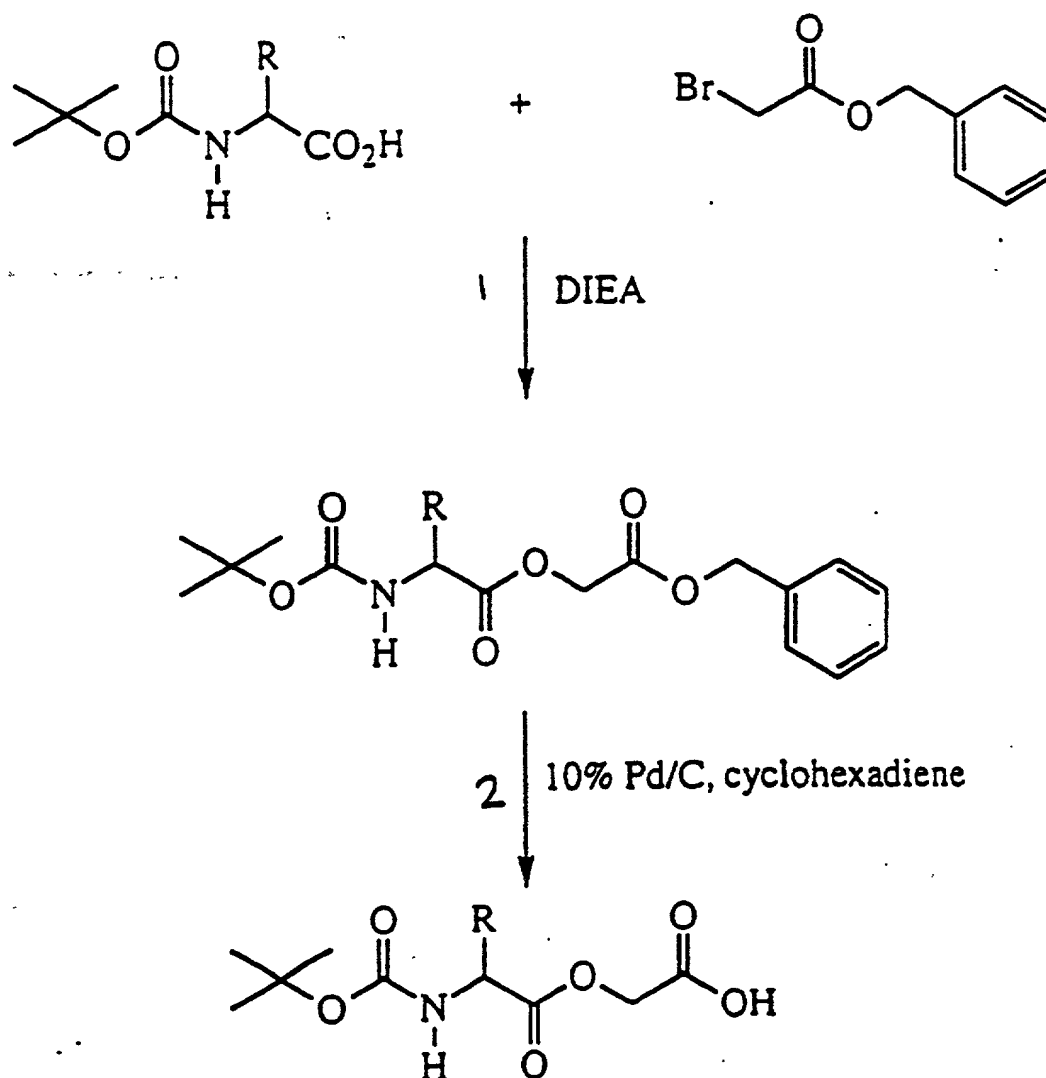


FIG. 23

Synthesis of C-terminal peptide segment

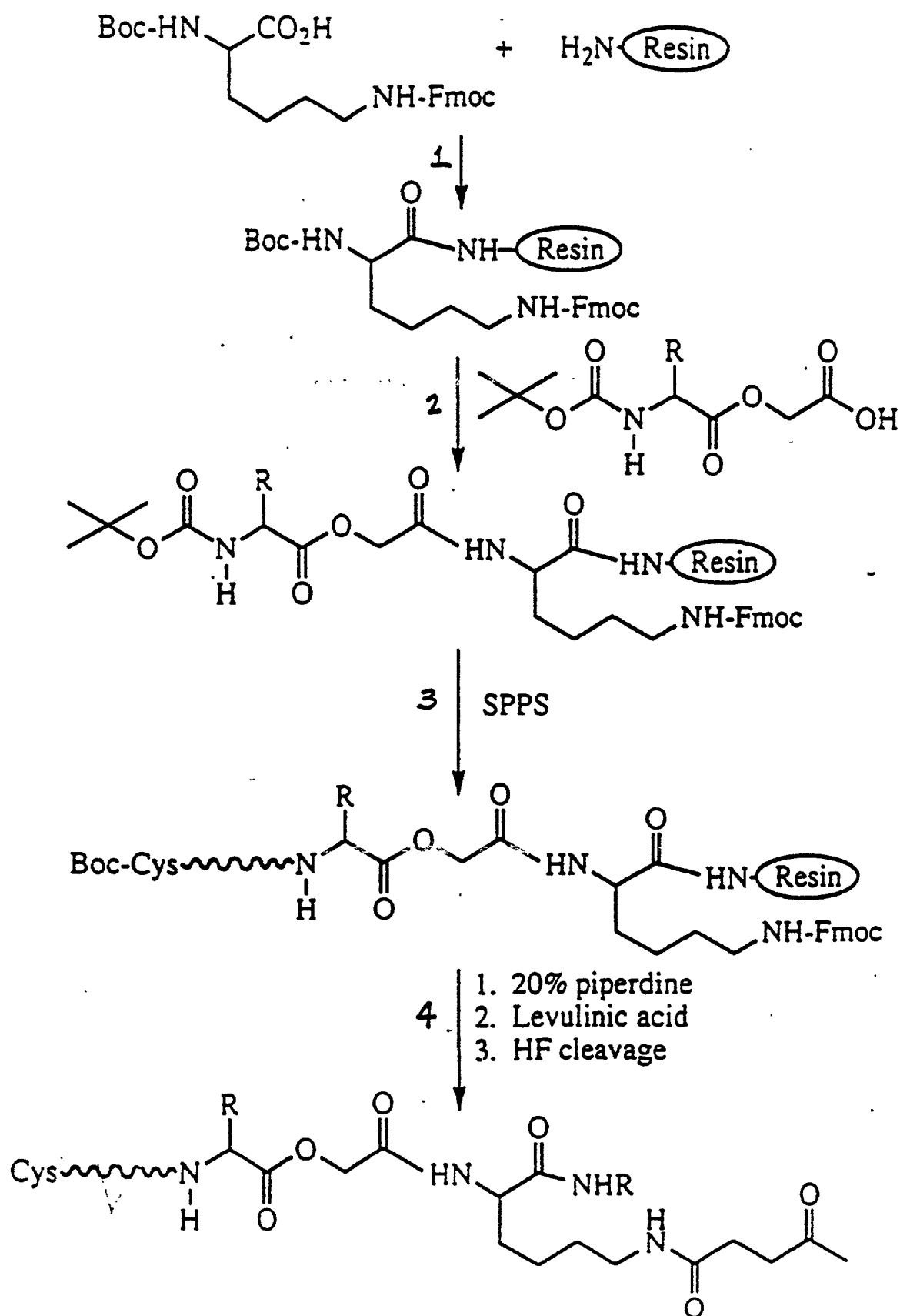


FIG. 24

Universal Solid Phase Chemical Ligation **(Bidirectional Ligations: C- to N-Terminal Ligations First)**

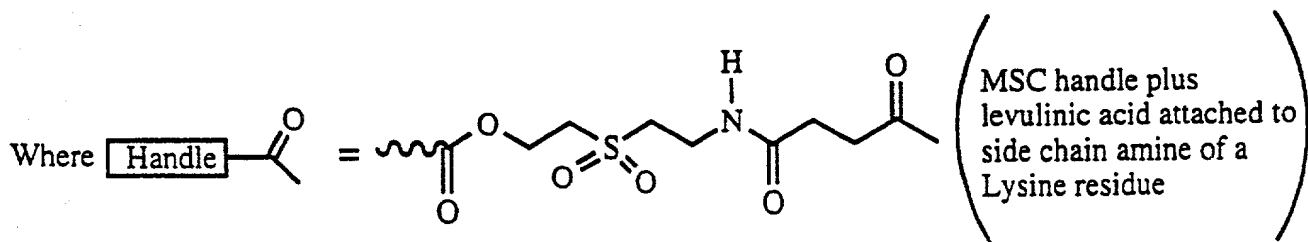
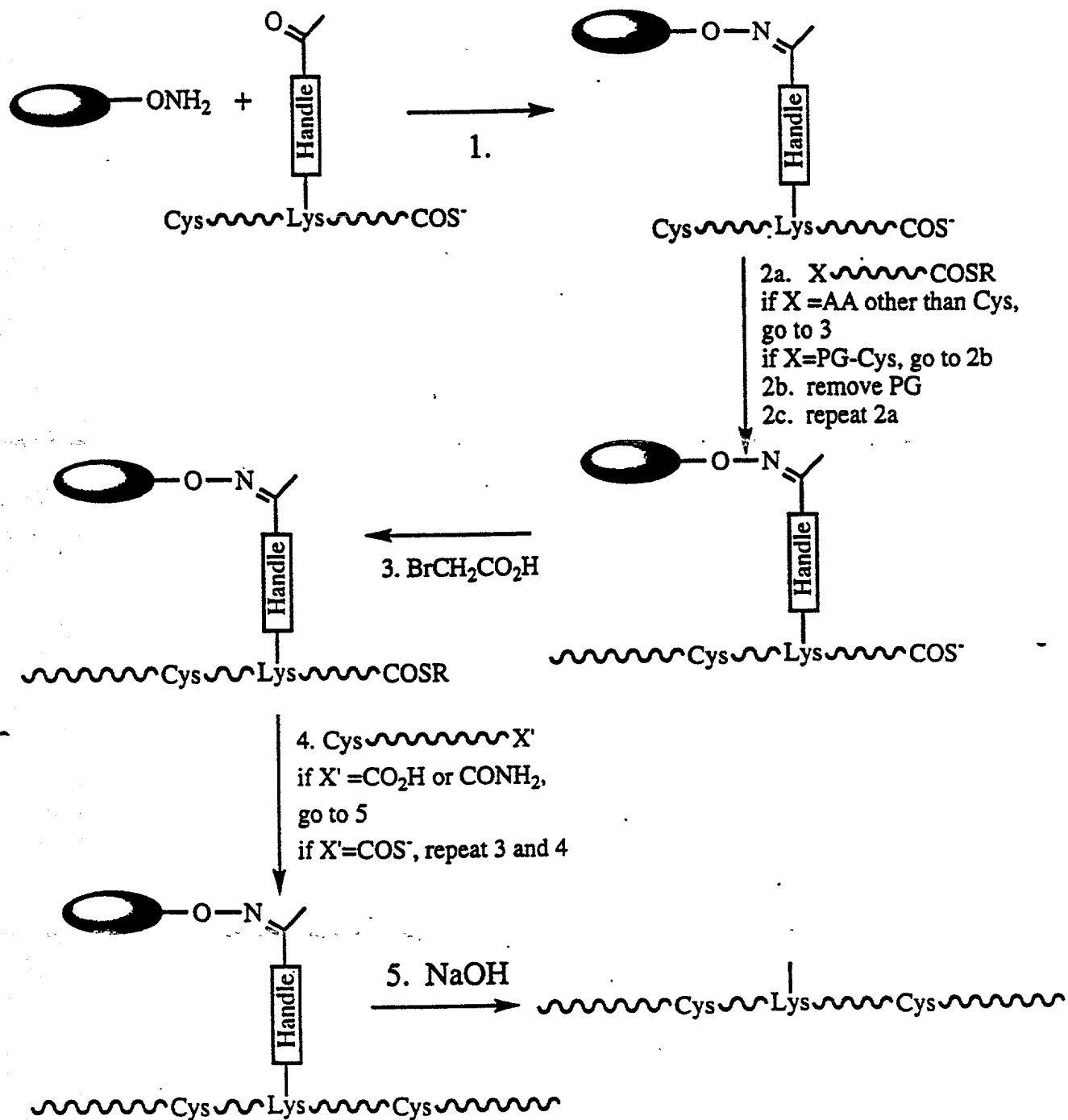
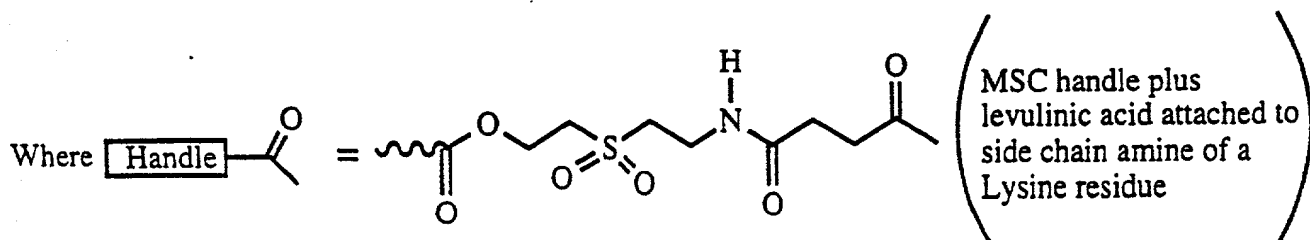
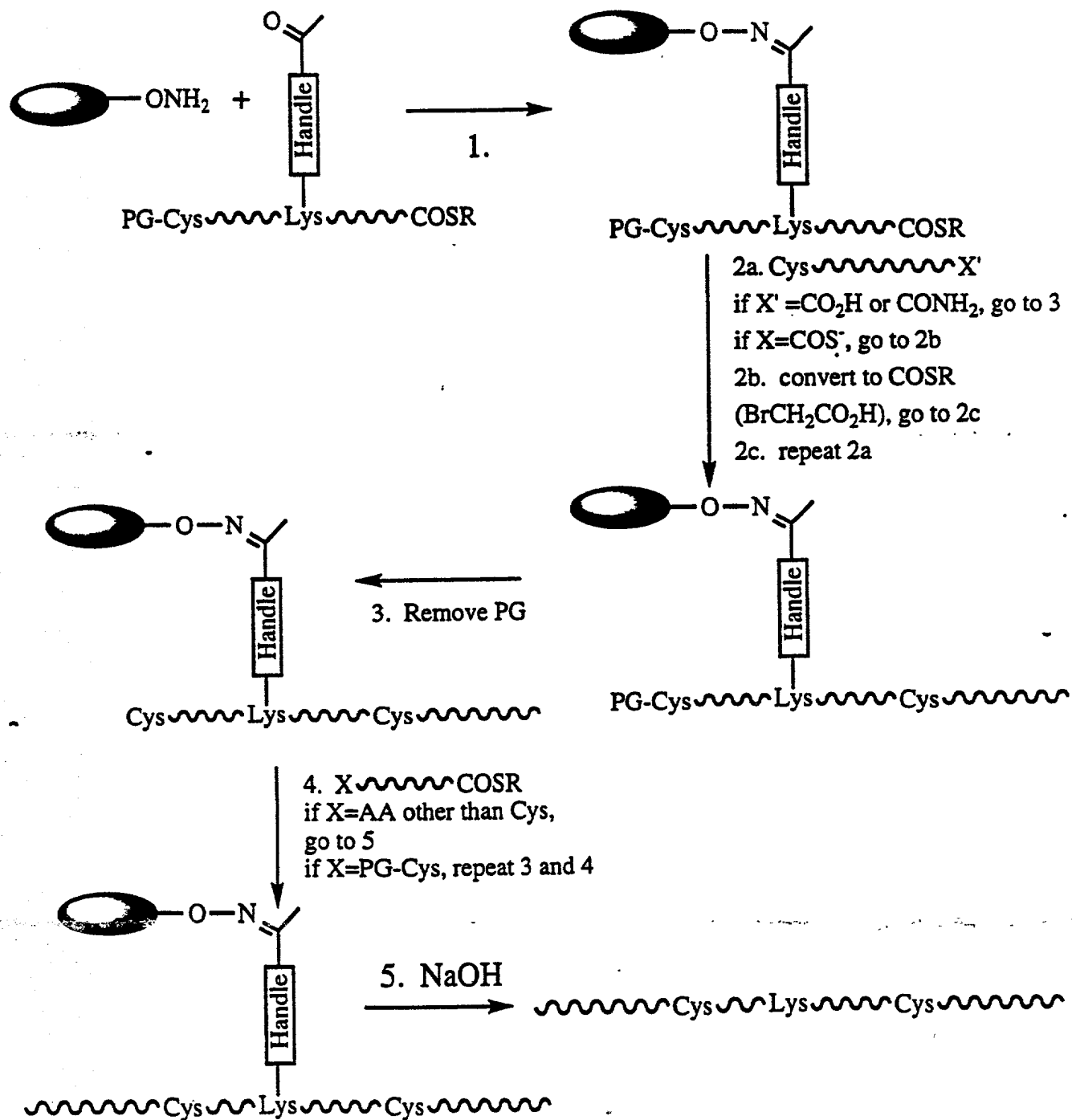


FIG. 25A

Universal Solid Phase Chemical Ligation **(Bidirectional Ligations: N- to C-Terminal Ligations First)**



Synthesis of Modified Peptide Segment for Universal Solid Phase Chemical Ligation

Starting with an appropriate resin (thioester or thioacid generating), synthesize the peptide using standard Boc protocols until the Lys residue of choice is reached. Couple a Boc-Lys(Fmoc)-OH, then continue the rest of the synthesis.

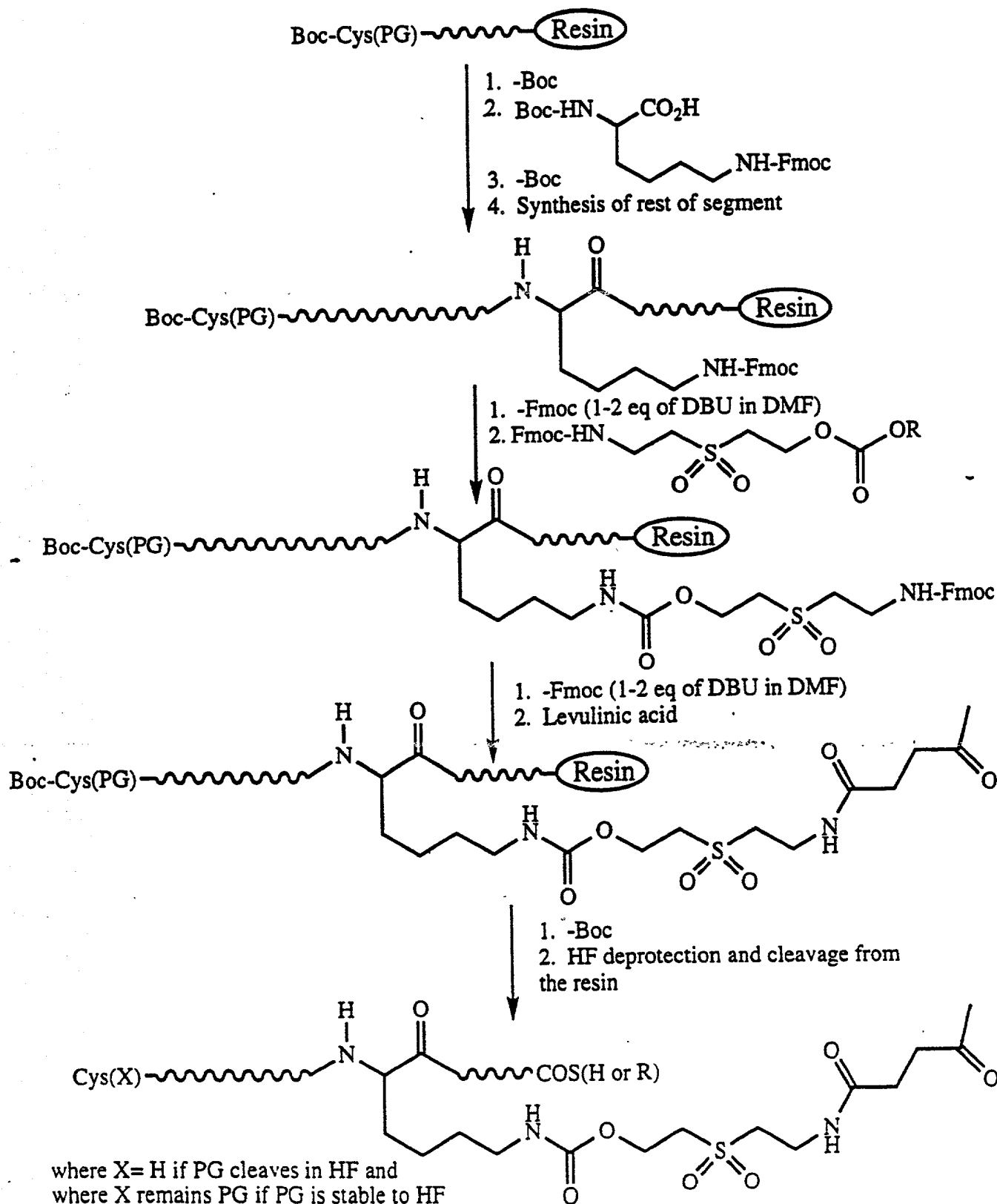
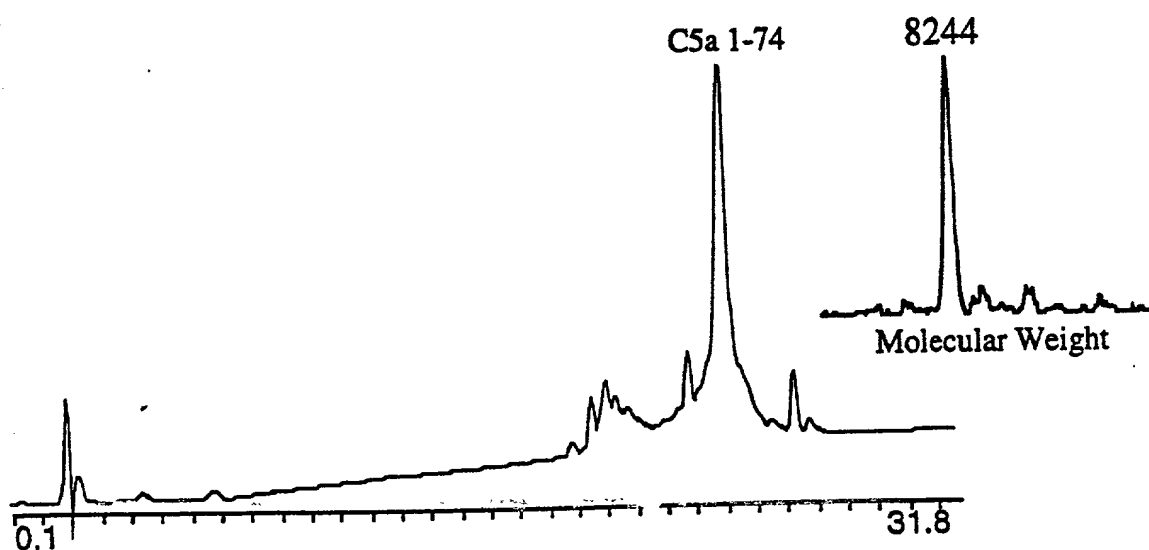
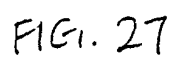


FIG. 25C

1 21 47
TLQKKIEELAAKYKHSVVKKCCYD GACVNNDETCEQRAARISLGPKCIKAFTECC
VVASQLRANISHKDMQLGR
74



[illegible]

**Synthesis of a Random Sequence by Solid Phase
Chemical Ligations in the C- to N-terminal Direction
Using Fmoc Protection**

ALTKYGFYGCYGRLEEKGCADRKNILA
1 10 19 27

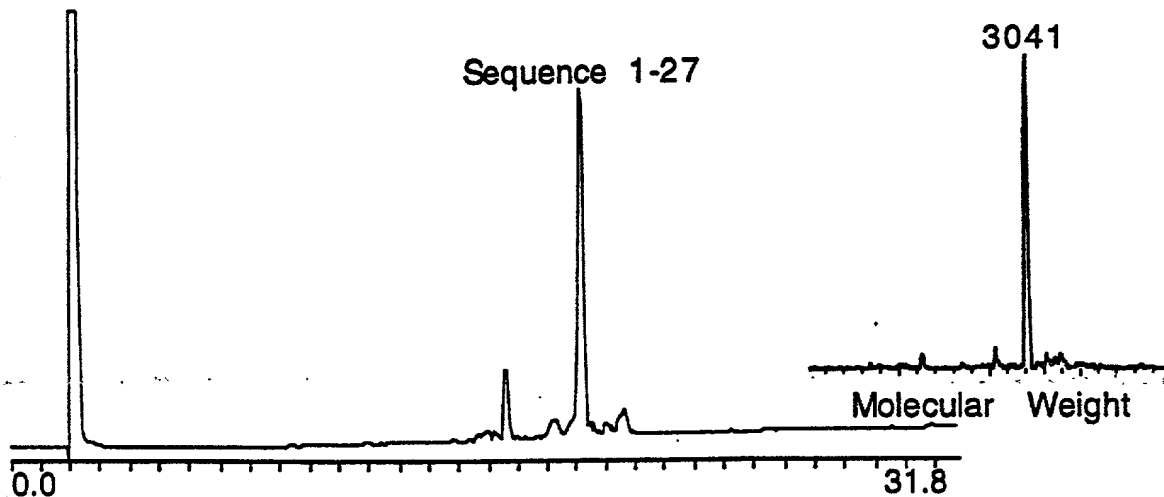


FIG. 28

Synthesis of a Random Sequence by Solid Phase Chemical Ligations in the C- to N-terminal Direction Using ACM Protection

ALTKYGFYGCYGRLEEKGCADRKNILA
1 10 19 27

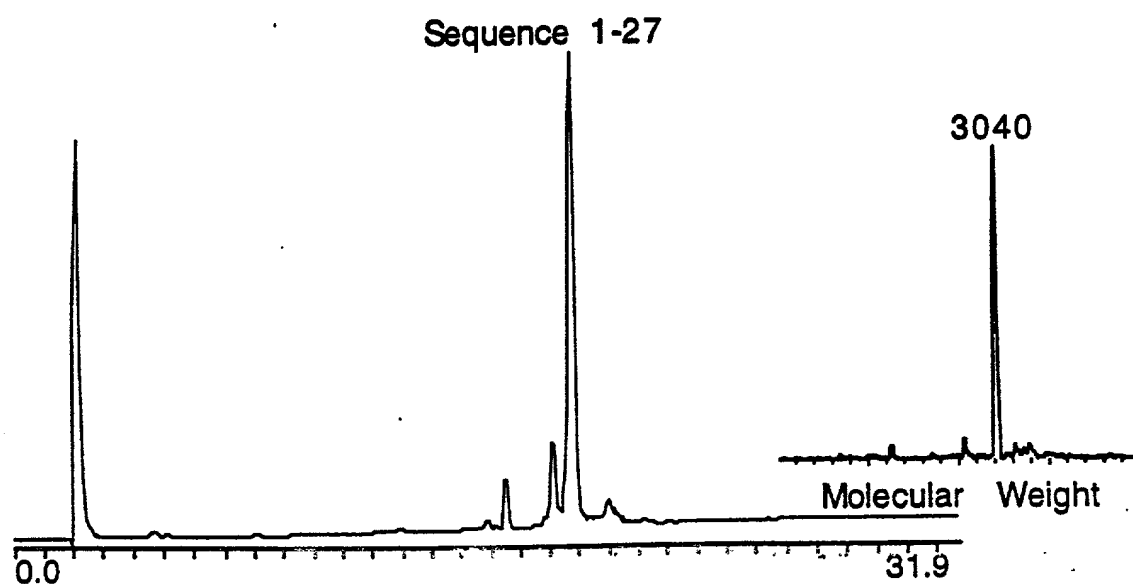


FIG. 29

Synthesis of Phospholipase A2 Group 5 by Solid Phase Chemical Ligations in the C- to N-Terminal Direction

1 26 59
 GLLDLKSMIEKVTGKNALTNYGFIYGCYCGWGGRTPKDGTDWCCWAHDECYGRLEEKGC
 NIRTQSYKYRFAWGVVTCEPGPFCHVNL**CACDRKLVYCLRNLR**SYNPQYQYFPN**ILCS**
 88 118

PLA2G5 88-118

3774 Da

Ligation 1

PLA2G5 59-118

7145 Da

Ligation 2

PLA2G5 26-118

10876 Da

Ligation 3

PLA2G5 1-118

13593 Da

0.0

46.9

Synthesis of Phospholipase A2 Group 5 by Solid Phase Chemical Ligations in the C- to N-Terminal Direction

1 26 59
 GLLDLKSMIEKVTGKNALTNYGFYGCYCGWGGRGTPKDGTDWCCWAHDECYGRLEEKGC
 NIRTQSYKYRFANGVVTCEPGPFCHVNLCACDRLVYCLKRNLRSYNPQYQYFPNILCS
 88 118

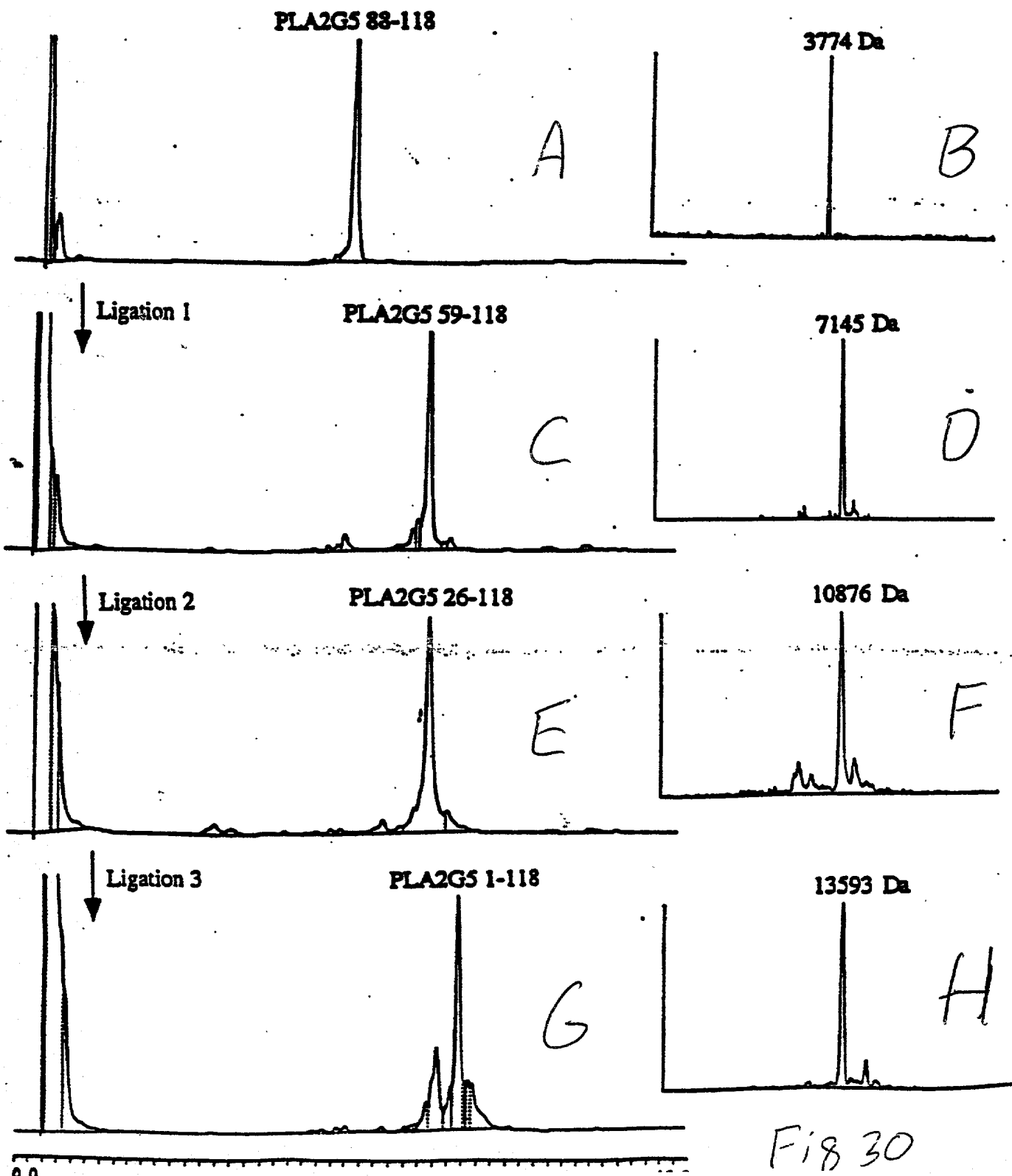


Fig 30